New species for EU aquaculture

Deliverable Report

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Deliverable Title	Methodology for meagre feeding		
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WP Title:	Grow out husbandry - meagre		
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Objective Development of an appropriate methodology for feeding of meagre during on-growing in cages

Description: For the development of an appropriate feeding methodology the following steps were implemented according to the description in DoW and adaptations applied during the course of the project.

The **first step** was to test **in tanks** whether meagre responds to **different feeding stimuli** (mechanical, optical, etc) and also to test **different feeding methods**. For this, groups of two different individual sizes (50-100 and 700-900 g) at different tank sizes (500 and 5000 l respectively) were used for testing mechanical and optical feeding stimuli for a period of 4 months (each group). Monitoring with video recordings allowed the definition of the optimal feeding stimuli. Three methods were tested with fish from two different ages (50-100 and 700-900 g) at different tank sizes (500 and 5000 l) for a period of 4 months (each group). The methods tested were: (i) Self feeder, (ii) Automatic feeding three times per day and (iii) Hand feeding. Monitoring with video recordings and records of the self-feeding activity were performed.

The **second step** was to test **in cages different feeding periods** related to the presence of light (day-night) and also **different feed distribution methods**. For this an experiment was implemented to test the performance of meagre in cages when fed exclusively either during the day or during the night. In a second experiment the feed distribution from the surface and from the bottom were compared. The tests were performed during two duplicated trials in the HCMR pilot farm in (6x6x8 m3) cages. One size group was used for a period of 8 months for each trial. Growth performance was estimated

with monthly samples while every second month haematological, biochemical, immunological and hormonal evaluation was performed. Also, the vertical distribution in cages will be monitored using an echo integrator.

The **third step** was the comparison of **automatic and demand type feeding in tanks** for a production cycle. Comparison in each season of the year of (a) demand feeding and (b) feeding with automatic feeders programmed to follow the feeding routines that are used customarily in meagre cage farms (feeding in farms is now based mainly of automated feeding). Three replicate control tanks (automated feeding) will be compared to three experimental demand-feeding tanks. Video cameras and sensors will be installed to register the activity of the fish and behaviours related to feeding and aggression. Experimental conditions will be natural photoperiod and simulated natural temperature controlled to be similar to sea cage growing areas for the specific season. The parameters to be evaluated would be: feeding time, feed delivered, growth, size variation in the population, FCR, pattern of fish activity, level of aggressive behaviours and fin condition.

All these steps provided sufficient information to propose a Methodology for meagre feeding.

Summary

The purpose of this work was to determine the optimum conditions for feeding meagre during growing out in cages with the aim of reducing size-variation and feed conversion ratio (FCR), which were identified as bottlenecks during grow out. Five experiments were completed examining different feeding systems, timing of feeding, surface or submerged feeding and feeding stimuli. In addition, two studies were made of the eye morphology and feed digestion times. Taken together the work demonstrated that normal cage feeding practices may not be major factors contributing to the high FCR or large size-variation that has been observed during grow-out. The experiments generated a large body of information that clearly identified the biological characteristics of meagre during grow out and thus give clear indications on how improvements can be made or mistakes avoided. Meagre were not an aggressive fish towards conspecifics. Low levels of fin damage indicated no aggression and eye morphology indicated a shoaling "social" fish. The non-aggressive meagre, appeared to be opportunistic feeders that fed during the entire 24-hour period, but especially during the night. There were no differences in growth, size variation or FCR when meagre were fed continually (self-demand feeding systems), during the night or day, hand fed or fed with automated programmed feeders, or fed at the surface or deep in the water column. However, meagre exhibited clear nocturnal habits. Feeding and activity was higher during the night, when meagre rose to the surface in both cages and tanks compared to staying at the bottom during daylight. Eye morphology indicated an organism adapted for nocturnal vision. Feeding and activity was suppressed by high light intensity. Meagre that were fed at depth had improved immune parameters compared to meagre fed during the day that rose to the surface during periods of higher light intensities. The digestive system evacuated in 8-12 hours at 19ºC. In addition to high light intensity, temperature (optimal temperature appeared to be 16-23ºC) and stocking density (optimal density appeared <20kg/m^3) also appeared to affect growth. Therefore, meagre are adapted to feeding at low light intensities and during grow out feeding should be aimed to coincide with low light intensity periods to provide meals separated by 8-12 hours (200 g fish at 19ºC). Although good results were obtained with other feeding programs or systems (e.g. continual self-feeding), feeding systems designed to address the biological characteristics described in the present study may improve repeatability of growth, size variation and FCR. Lastly, meagre quickly learned and responded to feeding stimuli (light and aeration) and these stimuli represent a tool that can be used to reinforce feed management to ensure good feeding when the fish cannot easily be observed.

1. INTRODUCTION

The meagre (*Argyrosomus regius*) 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., 2013a; 2013b; 2015; 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 7 fold (FAO). In 2016, meagre European aquaculture production was 7,280 t, produced in Spain, Greece, Turkey, France, Portugal, Italy, Cyprus and Croatia (FAO, 2018).

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 tends to stay in the bottom of the cage, feed low in the water column and take time to rise towards the surface to feed. The current feeding management is primarily based on feeding tables that have been designed based on the age and the size of each species and the rearing temperature. Feeding is performed either with automatic programmed mechanical systems or by hand.. In order to develop a proper feeding method a knowledge base for the meagre is required in order to design a proper species-specific feeding system. Parameters including the possible response to stimulus, preference on feeding methods, behavioral and feeding patterns in tanks and cages are critical for selecting appropriate methods but are lacking for meagre. In the work presented here some of these parameters were studied and the related results are presented.

For the development of an appropriate feeding methodology for a new candidate species, knowledge of feeding behaviour and the specific biological rhythms of the rearing fish is required. The fish have the ability to recognize different stimuli, connecting them with different events and adapting special behavioral patterns accordingly. Furthermore, when fish are repeatedly exposed to a stimulus associated with reward or punishment, they will develop a conditioned response (Folkedal, et al., 2017). These reactions have been demonstrated in numerous fish species relevant for farming and research such as the zebrafish *Danio rerio* (Manabe, et al., 2013), the rainbow trout *Oncorhynchus mykiss* (Colson, et al., 2015; Nordgreen, et al., 2010), the Atlantic salmon *Salmo salar* (Bratland, et al., 2010), the Atlantic cod *Gadus morhua* (Nilsson, et al., 2008a; b) and the Atlantic halibut *Hippoglossus hippoglossus* (Nilsson, et al., 2010). Signalization of food arrival could be used in everyday farming to make feeding more predictable and to assess the appetite in fish groups (Bassett and Buchanan-Smith, 2007; Fernö, et al., 2011). Signaling may advertise not only what is going to happen, but also where, and reward conditioning can be used to lead fishes to a feeding area (Midling, et al., 1987). As, no experiments have been performed on **the effect of different stimuli on the feeding behavior in meagre this represent one objective of this study**. Additional work focused on the histological analysis of the meagre eye and especially on the retina was performed as eye is the organ related with the optical stimuli under study.

During feeding the special behavioral characteristics of the reared organism ought to be harmonized with the methodologies used. Main parameters are the feed distribution frequency and the daily feed ration that should be analyzed in correlation with the food characteristic as the chemical composition of feed, the energy caloric content, the size and the shape of pellets, the colour and the texture

(Higuera, 2001). Moreover, not only the quantity and the quality of feed but also the method employed to deliver it have to be determined. Feed delivery could be manual, automated or demand feeding (Alanärä, et al., 2001). However, demand feeders, other than effective feeding methodology, also offer useful scientific information about the feeding activity of the fish. There is limited information for meagre and the aim of the second experiment was to **evaluate three different feeding methodologies** i.e. self-feeder, programmed feeding and hand feeding. Additionally, a study related with the evacuation rates of the digestive canal will offer more information for the development of the appropriated methodology for meagre feeding management.

As mentioned before, meagre presents a particular behavior during cage rearing, being concentrated at the lower layers of the cage responding thus to the benthic character of the species. Based on the results obtained in previous trials (see also Deliverable 20.2) meagre exhibited a particular nocturnal behavior by changing the vertical distribution of the group within the cage volume. In order to test whether this spatial behavior is also related to changes in feeding behavior the exclusive feeding during day or during night was studied. Further to the previous trials on feed delivery methods we evaluated during cage rearing two feed distribution methods from the surface and submerged. The trials were implemented in order to **define a proper distribution method in terms of period and location** in the water column of a cage.

A survey of meagre producers conducted in the preparation of the Diversify proposal found that a bottleneck in the production of meagre was variable growth rates during grow-out. A multidisciplinary approach is required in order to examine the role of genetics, nutrition --particularly dietary requirements during weaning, pre-ongrowing and in cage culture-- feeding behaviour and grow out husbandry. In meagre, despite of the great growth potential, both growth and feed utilization rates may be poor (Chatzifotis et al., 2010; Estévez et al., 2011) and feed delivery may be part of the problem. Meagre feed deep in the cages, which makes it difficult to assess feeding behaviour and, therefore, difficult to improve feed delivery and up-take. Demand feeding has been demonstrated to improve growth rates, improve food conversion ratio (FCR), reduce variation in size and reduce aggression in Atlantic salmon (Noble et al., 2007, 2008). Whilst for European seabass growth and FCR were the same or improved with no difference in size variation and aggression was not monitored (Boujard et al., 1996; Azzaydi et al., 1998, 1999, 2000). In seabass improved growth and lower FCR was observed in both studies conducted in cages (Azzaydi et al., 1998) and tanks (Azzaydi et al., 2000), whilst in salmon results in different cages all improved growth and FCR. Although demand feeding studies were conducted in tanks for salmon (Kadri et al., 1997) and control groups without demand feeding were not included as a comparison, Noble et al., (2008), demonstrated that the results from demand feeding trials could be used to improve feed tables for fixed automated feeding to obtain the same growth and FCR as obtained with demand feeding in tanks. Defined feeding rhythms were found in both species, salmon (Kadri et al., 1997; Blyth et al., 1999) and seabass (Azzaydi et al., 2000, 1999, 1998), although this was related to season and stage of development. These feeding rhythms were similar in tanks (salmon: Kadri et al., 1997 and seabass: Azzaydi et al., 2000, 1999) and cages (salmon: Blyth et al., 1999 and seabass: Azzaydi et al., 1998). Work on seabass demonstrated that automated feeding in relation to feeding rhythms compared to feeding equal ratios throughout the day gave the same or improved growth and lower FCR (Azzaydi et al., 2000, 1999). Altogether these results show that demand feeding research in cages and tanks gave similar results and has be used to improve feed tables and feeding regimes to reduce size variation, improve growth and FCR in fish held in cages. The present sub-task aimed to apply this approach to meagre in tanks for the use in standard automated cage feeding systems.

The objective was to acquire the necessary information for the development of appropriate feeding methodologies for cage culture of meagre, in order to maximize the performance.

2. MATERIALS AND METHODS

2.1 The effect of stimuli on the feeding behavior of meagre

Three consecutive experiments took place at the facilities of the Institute of Marine Biology, Biotechnology and Aquaculture at the Hellenic Center for Marine Research (HCMR) in Crete, Greece. The first two were performed indoor with individuals ranging between 50-100 g in body weight while the third was performed outdoors with individuals ranging between 600 to 900 g.

2.1.1 The effect of stimuli on the feeding behavior of meagre with body weight 50-100 g

Two Experiments were performed (Exp1 and Exp2), with individuals of body weight between 50 to 100 g in 500-l tanks. Fish of the experimental groups were reproduced at HCMR.
The experiments were performed in 500 l indoor tanks in triplicates. Each triplet of tanks was organized as a semi-closed recirculated system with controlled water temperature. The photoperiod was in correspondence to the natural one (35°20′N, 25°08′E from February to June). The diet of the fish throughout the experiment was industrial feed pellets distributed with an automatic feeder.
In Exp1, which lasted for 40 days, the effect of stimuli on the feeding behavior of a naive population, as concerning the previous experiences with the specific stimuli, was studied. In Exp2, which was performed one month later, the same experimental populations of Exp 1 were used.
Four experimental groups were used related to the stimulus used. For the first group, called ‘’Light’’, the stimulus was a fading light, coming from waterproof LED lamps that were placed in the water column 10 cm below the water surface under the feeder used. For the second group, called “Air”, the stimulus was air bubbles released from a 5-mm plastic tube that was placed at the same position as before under the feeder. For the third group, called “Air and Light”, a combination of the previous stimuli was used, ‘but the “Light” stimuli were performed during morning and afternoon while the “Air” stimuli during noon. The fourth experimental group was the control group with no stimuli before feeding.
In order to monitor the behavior of the fish a recording camera was placed above each tank (**Figure 2.1.1.1**). All data collected using the “Geovision” software was stored in a hard disc for further analysis.

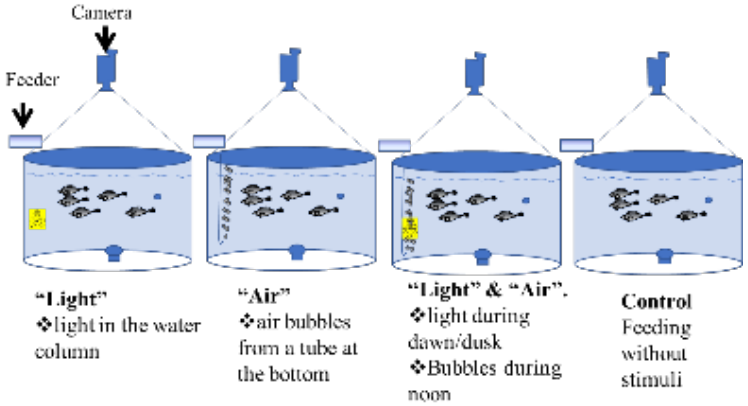

Figure 2.1.1.1. A schematic representation of the experimental tanks with the different types of stimuli.

The feeders were activated three times per day. At 08:30 in the morning, at 12:30 at noon and at 16:30 in the afternoon. First, the stimulus was applied, which had a total duration of 45 sec. At 40 sec the feeder was activated and the feed pellets were released at the feeding area. A schematic representation of the process is shown in **Fig. 2.1.1.2**.

The analysis was performed in 5-minute videos, which were recorded at 11 different times during the day. More specifically, videos were recorded during the three feeding periods, 15 minutes before and after the feeding periods and at 10:30 in the morning and 14:30 in the afternoon (**Fig. 2**). Moreover, each 5-minute video during the feeding period was divided into 2 periods, the 4 min and 15 sec-period without stimuli and the 45 sec-period when the stimuli and the feeding were performed .

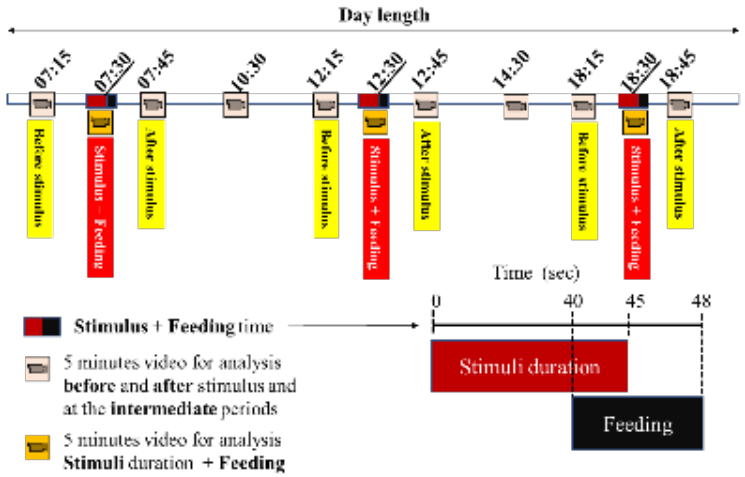

Figure 2.1.1.2. Schematic representation of recording periods during the day and analysis of stimuli.

For analysis, the Kinovea software (v.0.8.15, 2006-2011) and ImageJ systems (ImageJ 1.50b, Rasband W., National Institutes of Health, USA, https://imagej.nih.gov/ij) were used. Initially each 5-min was divided in 70 consecutive time intervals and from each one a picture was extracted (using Kinovea). In each picture the place of the feeder and the head of each fish were marked and the corresponding coordinates from these points were extracted using image J. After some basic trigonometry (**Fig. 2.1.1.3**) the distance of each fish from the vertical position of the feeder was calculated. The mean distance of the fish population from the feeder for each picture was calculated using the values corresponding to the distance from the feeder (in pixels) of the head of each fish. The previous process was applied to all 70 images in each 5-min video that was analyzed.

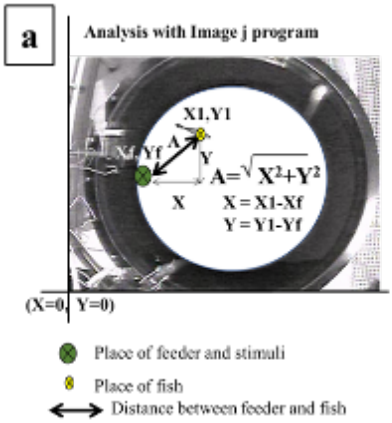

Figure 2.1.1.3. Schematic representation a) of the calculation of the distance between the fish and the feeder using the coordinates of the feeder and the fish as they are extracted by image J

2.1.2. The effect of stimuli on the feeding behavior of meagre with body weight 600-900 g

The experiment was conducted during two months (September - October 2015). Six outdoor tanks with a capacity of 10 m^3 were used, duplicated for each of the three experimental groups. Ten one-year-old individuals of meagre naïve in any type of stimuli, with an average weight of 636.16 ± 56.56 g were placed in each tank. The diet of the fish throughout the experiment was industrial feed distributed with a feeder.

Based on the results of Exp 1 and 2, three experimental groups were created. For the first, called ''Light'', the stimulus was a fading light, from a waterproof ribbon of LEDs, placed in the water column 10 cm below the water surface under the feeder used. The maximum light intensity of the lamps, as it was measured from a distance of 40 cm, ranged between 350-400 lx. The intensity of the natural light at noon with sunshine had a value from 24,000 lx to 34,000 lx. For the second group, called "Air", the stimulus was air bubbles released from a plastic 5 mm tube placed at the same position in the water column as the light stimulus. The third experimental group was the control group with no stimuli before feeding.

The same schedule regarding the feeding times and the recording periods as in Exp1 and Exp2 was followed (**Fig. 2.1.1.2**). From the total 60 experimental days, 14 days were selected for the analysis, one every 4 days. Following similar procedures as presented for Exp1 and 2, from each 5-min video, 70 pictures were extracted and analyzed and the coordinates of each fish (the head) in the tank was determined. In particular, each tank was divided into six equal square areas (**Fig. 2.1.2.1**).

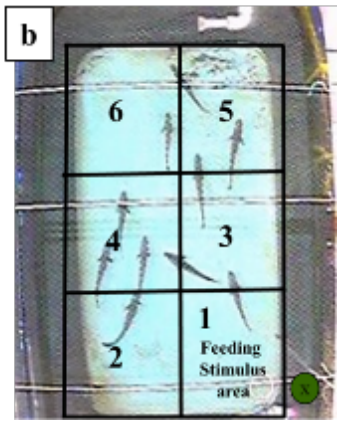

Figure 2.1.2.1. Representation of the different areas that tanks were divided. Area 1 included both the stimulus and feeding area, whilst the green circle represents the position of the feeder.

Based on the coordinates of each fish its location in one of the six areas was determined. Subsequently, the distribution of the individuals in the tanks at different times was calculated.

2.1.3. Histological analysis of meagre eye.

For the histological analysis, 3 pairs of eyes were removed from three individuals of meagre that had a mean weight of 200 ± 20 g. The eyes, before being embedded in methacrylate resin (Technovit 7100®, Heraeus Kulzer, Germany), were dehydrated in gradually increasing concentrations of ethanol solutions (70-96%). Serial sections of 3 μm were obtained with a microtome (RM2245, Leica, Germany). Sections from the retina were stained with Methylene Blue (Sigma, Germany)/Azure II (Sigma, Germany)/Basic Fuchsin (Polysciences, USA).

Six different areas from the retina of each eye were used for the quantitative determination of the different cell types. Photographs were taken from histological sections using a digital camera mounted on a microscope at different magnifications. Thereafter, using an image-analysis software (Image J, National Institutes of Health), a number of different 100-μm regions of the retina were examined.

The parameters that were measured were: the number of cones at the photoreceptor layer (PL), the rods that were counted based on the number of nuclei of the rods (RN) at the outer nuclear layer (ONL), the cells at the inner nuclear layer (INL) and the ganglia cells (GC) at the ganglion cell layer (GCL), in 100-µm sections of the retina. Using the number of rods and cones, the number of cells in the INL and ganglia cells were calculated. Also, the ratios of rods to cones, rods to ganglia cells, ganglia to INL, rods to INL and cones to ganglia cells were calculated.

2.1.4. Statistical analysis

The differences of the mean distances (Exp1 and Exp2) of the fish at the different periods of the day were analyzed using analysis of variance (ANOVA) and the statistical significance was set to 95% ($P<0.05$) using the Sigma stat 3.5 (Systat Software Inc., USA) statistical package.

2.2 Test of different feeding methods

The different feeding methodologies were tested in two experimental groups of meagre differing in size. The fish used for these experiments were obtained from a broodstock that reproduced in captivity at the Institute of Marine Biology, Biotechnology & Aquaculture Hellenic Center for Marine Research.

2.2.1 Feeding methodologies tested

Three different feed delivery methodologies were tested in triplicates. In the first, the feed delivery method applied was by hand and fish were fed 3 times a day (8:30, 12:00 and 15:30) ad libitum. The second method tested was the use of automatic feeders, activated 3 times per day (08:30, 12:00 and 15:30). The released quantity of food in each feeding corresponded to 0.5% of the total biomass in each tank. The third feed-delivery method tested was the demand-feeding. The self- feeders used consisted of an immersed lever at a depth of 30 centimeters from the surface (total tank depth 60 centimeters for indoor tanks and 170cm for outdoor tanks). When the lever was moved from its equilibrium, an electrical signal activated an automatic feeder and the feed was released. Each activation was, wireless, recorded and the total activity was monitored across the 24-hour period (extracted at three-hour intervals), throughout the experiment.

Above each tank a recording camera was placed in order to record the distribution of the fish. All the data were stored in a hard disc for further analysis. The recording program that was used was the "Geovision". For video analysis, the Kinovea video analysis program (v.0.8.15, 2006-2011) and ImageJ systems (ImageJ 1.50b, Rasband W., National Institutes of Health, USA, https://imagej.nih.gov/ij) were used.

The feeding methods were tested with two different size classes of fish as explained below. A third trial was performed to estimate the digestive evacuation rate of meagre.

First trial (EXP1). Individual size of 50-100 g (in 500 l tanks)

The first trial was conducted in black indoor tanks of 500-l, connected to an open circuit system with a total water renewal of 400% per hour. The water used was natural sea water (38 psu) pumped from a littoral well. The water temperature was 19°C while oxygen saturation was above 75%. The photoperiod was natural corresponding to a geographic width of 35°N from May to August. An additional light coming from fluorescent lamps (450 lx) was used from 08:00 to 18:00. The fish density was 12 fish per tank. Fish were sampled every 4 weeks, and individual measurements of weight (g) and total length (cm) were taken.

Second trial (EXP2). Individual size of 700-900 g (in 5000 l tanks)

The second trial was conducted in black outdoor tanks of 5000-l, connected to an open circuit system with total water renewal of 400% per hour. The water used was also natural sea water (38 psu) pumped from the same littoral well. Water temperature was 19°C while oxygen saturation was above 75%. The photoperiod was natural corresponding to geographic width of 35°N from May to August. The fish density in this case was 11 fish per tank. Fish were sampled every 4 weeks, and individual measurements of weight (g) and total length (cm) were taken.

Third trial Digestive evacuation rates

For studying the evacuation rate of the digestive canal, an additional trial was performed with fish having body weight between 160 ± 20 g. The trial was performed at 19 °C. Before the experiment fish were acclimated for 1 month at a constant temperature of 19 °C. For the trial fish were anesthetized, weighed and then force-fed by introducing a number of pellets (corresponding to 0.5% of the body weight), with a special piston, directly into the stomach of each fish (Chatzifotis, et al., 2005). The study related with the evacuation rates of the digestive canal will offer the necessary information for the development of the appropriated methodology for meagre feeding management

2.2.2 Statistical analysis

For the evaluation of fish growth and feeding performance, the following indices were used: the specific growth rate (SGR), the feed conversion ratio (FCR), the daily feed consumption (DFC) and the condition factor (CF).

- Specific growth rate = $100 \times (\ln W_f - \ln W_I)/T$
- Feed conversion ratio = $W_{TFS}/(W_f - W_I)$
- Daily feed consumption = $W_{TFS} \times 100/[((B_I + B_f)/2) \times T]$
- Condition factor = $W_b/L_b^3 \times 100$

Where W_b is the body weight and W_I, W_f are the initial and final body weight (g) respectively, L_b is the total body length (cm). B_I and B_f are the initial and final biomass (g) respectively, while W_{TFS} is the weight of total dry feed supplied (g) and T is the duration of the experiment (days).

Data were analyzed by two-way analysis of variance (ANOVA) and the Student–Neuman Keuls' multiple range test to distinguish differences between treatments means (SigmaStat, Systat Software, Point Richmond, CA, USA). The level of significance chosen was $P < 0.05$ and the results are presented as mean ± standard deviation (SD).

2.3 Test of daily vs night feeding in the performance of meagre

The specific objective of the experiment was to test the performance of meagre in cages when fed exclusively either during the day or during the night. Cages of 290 (6x6x8) m^3 at the HCMR pilot farm in duplicates were used.

Fish origin was the hatchery of HCMR. Eggs were from a single spawning and larval rearing was performed at the Mesocosm hatchery of the institute. Juveniles of 2 gr were transferred at the cage facility and they were reared under similar conditions until the beginning g of the trial.

Four groups were created, of ~1,820 individuals for each cages.

The initial wet body weight at the beginning of the trial was 500 ±20 g. The duration of the trial was planned to be 8 months. It actually started on June 2016 and was completed on March 2017.

The temperature profile during the trial is presented in Figure 2.3.1

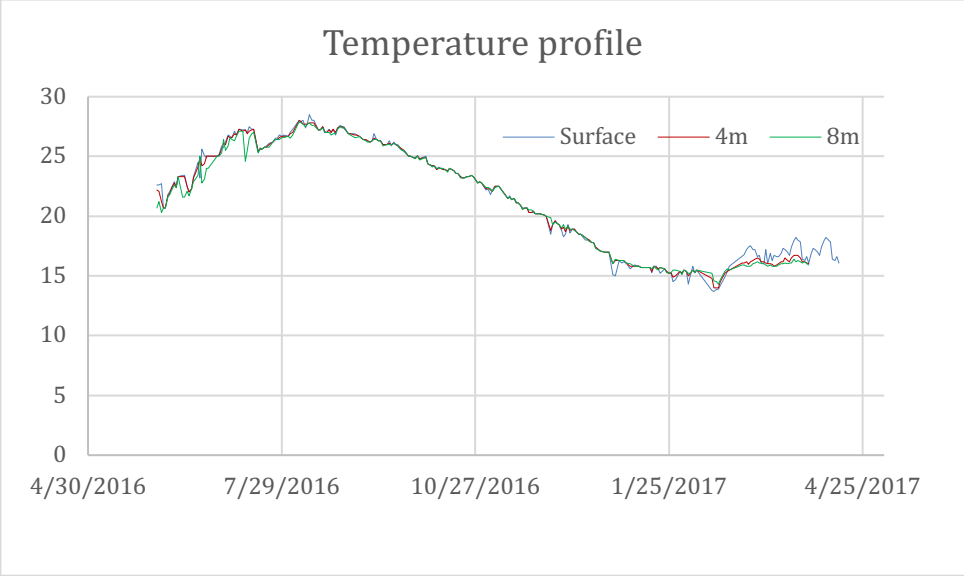

Figure 2.3.1 Temperature profile (surface, 4m and 8m depth) during the experiment

Feeding was performed with automatic feeders in half hour time intervals and the total amount delivered was similar (proportional to the biomass) in all cases. For the cages fed during day feed distribution started at 7:00 in the morning and finished at 18:00. The cages fed during night were gradually adapted to this change during a period of one month as it is shown in the following schematic representation. The feed distribution for the night feeding started at 19:00 and finished at 06:00.

	7:00	8:00	9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00	21:00	22:00	23:00	0:00	1:00	2:00	3:00	4:00	5:00	6:00
Day feeding																								
+1 week																								
+2 weeks																								
+3 weeks																								
+4 weeks																								
Night feeding																								

During the experimental period, growth performance was estimated with monthly samples. Every second month blood samples were taken for haematological (hematocrit, hemoglobin), biochemical (glucose, lactate)[1], and hormonal (cortisol) evaluation.

[1] The immunological (lysozyme, myeloperoxidase serum) analysis initially considered, were not performed due to a problem during sample transfer that resulted in the loss of all related samples.

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). Statistical comparisons of total length, body weight and the haematological, 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 were 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). For lactate measurements a commercial kit was also used (SPINREACT).

*Hormonal analysis***.**
For the determination of cortisol, plasma samples were extracted with diethyl ether and water samples were extracted according to Ellis et al. (2004) using ethyl acetate. 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).

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

2.4 Test of two feed distribution methods on the performance of the meagre in cages

The objective of the trial was to test two feeding distribution methods that was from the surface of the cage and using a submerged distribution device. The trial was performed at the pilot scale installations of HCMR in duplicates using cages of 290 (6x6x8) m^3 indicated as Normal and Submerged.
Fish origin was the hatchery of HCMR. Eggs were from a single spawning and larval rearing was performed at the Mesocosm hatchery of the institute. Juveniles of 2 gr were transferred at the cage facility and they were reared under similar conditions until the beginning g of the trial.
Four groups were created, of ~2,720 individuals for each cages.
The initial wet body weight at the beginning of the trial was 290 ±20 g. The duration of the trial was planned to be 8 months. It started in May 2017 and was completed on December 2017.
Feeding was similar in all cases (proportional to biomass of each group) but the distribution was different according to the condition tested. The standard feeding with feeders located on the surface of the cage was compared with feed distributed submergible. The submerged feeding was performed by transferring feed together with sea water through a flexible tube from the surface to a predefined depth approximately the medium depth of the cage (i.e. 4m). An electric pump located on the platform pumped water into the cage while an electric dosing mechanism delivered the required feed quantity (Fig 2.4.1).

Figure 2.4.1 Feeder installed during the trial for the submerged feeding

The feeding pipe was installed at the centre of the cage. A rotating S-form ending ensure an even distribution of the pellets (**Fig 2.4.2**).

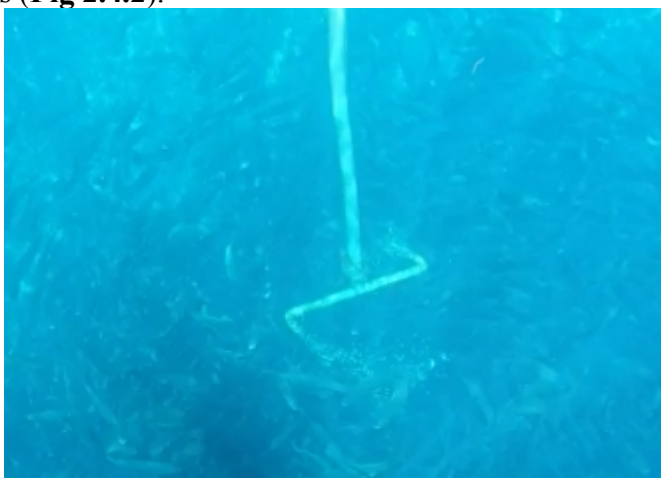

Figure 2.4.2 The S-form ending of the submerged feeding system

The temperature profile during the rearing is presented in **Figure 2.4.3**

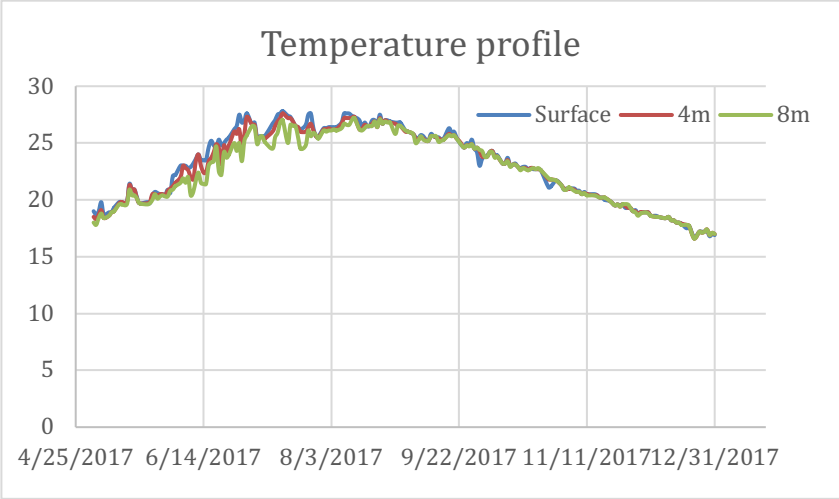

Figure 2.4.3 Temperature profile (surface, 4m and 8m depth) during the experimental phase

During the experimental period, growth performance was estimated with monthly samples. Every second month blood samples were taken for haematological (hematocrite, hemoglobin), biochemical (glucose, lactate), 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). Statistical comparisons of total length and body and also of the haematological, 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 were 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). For lactate measurements a commercial kit was also used (SPINREACT).

*Hormonal analysis***.**
For the determination of cortisol, plasma samples were extracted with diethyl ether and water samples were extracted according to Ellis et al. (2004) using ethyl acetate. 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 meager, *Argyrosomus regius*, were adapted from methods routinely used in the sera of Gilthead seabream or European sea bass (see also Deliverable 20.2).

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. The 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, *Argyrosomus regius*. 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. Results are expressed as units/ml of serum.

Despite not included in the original DOW of WP20, 2 supplementary parameters were investigated: The serum anti-protease activity was measured as described before for shi drum, *Umbrina cirrosa* (Henry and Fountoulaki, 2014). Results were expressed as the percentage of trypsin inhibition calculated using the standard curve as a reference. Ceruloplasmin oxidase activity which is considered to be a marker of the inflammatory response, was measured following the previously described method (Dunier et al., 1995) using 10µl of serum incubated with 100µl of the 0.1% para-phenylenediamine solution. The kinetic of increase of absorbance was followed at 550nm for 15min and 1 unit was defined as an increase of OD of 0.001/min (Henry and Fountoulaki, 2014).

Behavioral monitoring

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

2.5 Comparison of automatic and demand type feeding in tanks.

2.5.1. Fish and holding conditions

The juvenile meagre were brought to IRTA from Andromeda Iberica on the 8 June 2016. The holding conditions prior to starting the trail were natural temperature and photoperiod with feeding to satiation or using demand feeding systems during a training period. It was necessary to grow the fish to 40-50g before training to use the demand feeding system was achieved. Prior to initiating the experiment 1200 juvenile meagre were "trained" to use the pendulum demand feeding system. The experiment started on the 4 October 2016. The 1200 trained fish were randomly distributed into six 1500 L tanks, 200 fish per tank. The tanks were connected in pairs to a recirculation system (IRTAmar®) (**Picture 20.5.1.1**). As the fish grow, biomass was removed from the tanks by reducing the number of fish per tank. On the 10 January 2017 the number of fish per tank was reduced to 150 and on 7 June 2017 the number of fish per tank was reduced to 75. The reduction in numbers was made as following, first 60 fish were randomly selected for sampling (weight, length and fin condition) and secondly fish also randomly selected were added to the 60 to obtain 150 or 75 counted fish. The remaining fish were removed from the experiment and the randomly selected fish were returned to the experimental tank. All tanks were fitted with a net to avoid fish jumping from the tank. The photoperiod was natural and the temperature regimen simulated the temperature of the seawater on the Mediterranean coast of Spain where meagre are grown out in sea cages (**Fig. 2.5.1.2**). The photoperiod was maintained with a clock that switched the lights on and off in relation to the natural photoperiod for the region (latitude: 40.628º longitude: 0.665º). Daytime illumination was provided by fluorescent daytime lights that provided 120 ± 12 lux at the water surface. A low light illumination from an white LED covered with a perforated aluminium foil was used at night to simulate moon or star light illumination. The illumination was enough to visually see the outline of the shapes of the fish, but did not register an intensity on the luz meter (0 lux).

Picture 2.5.1.1. Experimental set up with six tanks connected in pairs to a recirculating aquaculture system (RAS, IRTAmar). Each pair of tanks were a programmed feeding regimen (Prog) and a auto-demand feeding system (AD).

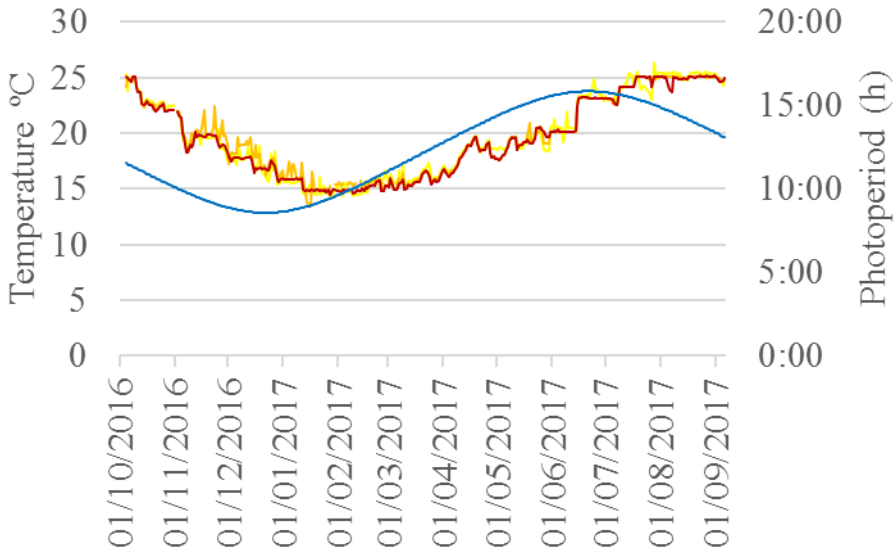

Figure 2.5.1.2 Temperature profile of the three recirculation systems, RAS 1 (yellow), RAS 2 (orange) and RAS 3 (red) and the photoperiod (blue) used for meagre (*Argyrosomus regius*) fed with two feeding systems, programmed and auto-demand.

2.5.2. Feeding

Three tanks were set up with the auto-demand feeder. The feeder delivered 5 g of feed when the pendulum was triggered, a demand. The food fell from the feeder within 0-5 seconds after the demand when the pendulum was triggered. The time of each feed demand was registered. Each day the feeder was filled with a pre-weighed excess of feed. Before filling the feeder, the previous days uneaten feed was removed and weighted to determine the exact amount of feed that was demanded. Three tanks were set up with programed feeders. The programmed feeders delivered three feeding periods (09:00, 13:00; 17:00) of 1 hour (feed ration was divided equally and delivered every 15 minutes during the hour) when the fish had a mean of 50-100 g and two feeding periods (09:00, 17:00) of 1 hour when the fish had a mean of 100-400 g. This follows cages farming feeding practices in Spain. The programmed amount of feed delivered followed manufactures feeding tables. The pellet size for both programmed and demand feeders was adjusted to fish size following manufactures recommendation. Each morning the tank bottom and tubes where faeces and uneaten food would collect were revised and the presence of uneaten feed was noted.

2.5.3. Fish Sampling and behaviour analysis

The fish were sampled on the days between 4-10 during the first half of each month. Each month a random sample of 60 fish from each tank were weighed, length measured and fin condition scored. The mean weights were used to calculate the specific growth rate (SGR) using the equation $SGR = ((\ln Wt_1) - (\ln Wt_0))/t \times 100$ where Wt_1 = weight at the end of the growing period; Wt_0 = weight at the beginning of the growing period and t = number of days of the growing period. The mean weights were used to calculate the feed conversion ratio (FCR) using the equation: $FCR = Ft/(Wt_1-Wt_0)$ where Ft = total food consumed or delivered to the tank; Wt_1 = weight at the end of the growing period; Wt_0 = weight at the beginning of the growing period.

A scale of fin condition was used to assess the fin condition, where 1 = perfect fins with no damage, 2 = light damage, 3 = excessive damage and 4 = no fin. The trail finished on 6 September 2017.

Each tank had two movement sensors that registered when a fish passed within ~35 cm in front of the sensor. One sensor was positioned 20 cm below the water´s surface and a second 70 cm below the water´s surface and 20 cm from the bottom of the tank. The activation of the sensors by a passing fish was registered in a computer during the entire experiment 24 hours a day. The data from three random days per month per sensor were used to analysis the behaviour of the fish.

During August 2017, the behaviour of the fish in each tank was recorded on four days. Behaviour was recorded with a GoPro camera (GoPro Hero 5 session, GoPro, California, USA) positioned at the water surface angled downwards (at 90º to the wall of the tank) to record the behaviour of the fish swimming around the tank. A period of five minutes was recorded at five time points during the day, 08:30, 10:30, 13:30, 16:30 and 18:30. In relation to the programmed feeding system this represented a recording half an hour before and after the two feeding periods and a recording at the middle of the day (between feeding periods). Recordings were made on four different days, the 2 Aug., 3 Aug., 16 Aug. and 17 Aug. The videos were observed for aggressive interactions (chasing or biting) between the fish and analysed to determine swimming velocity. To determine swimming velocity the videos were analysed with Fiji (www.fiji.sc) using the plugin Kymograph (written by Rietdorf and Seitz) complemented with velocity measurement tools included in the same plugin. For each five minute video, two periods of 300 frames (10.3 seconds) were analysed. During the analysis of each period of 300 frames the field of capture was divided into three areas (right, central and left area of the video frame) and each area was analysed. The program tracked reference points in each area that represented the trajectory of the swimming group of fish and calculated the velocity of each tracked point over the video frames. The velocity of each area was calculated (the mean of the points) and the mean of the three areas was calculated to represent the swimming speed of the 300 frame video.

Video recording was also made during one week in February 2017 with red night illumination to observed demand feeding during the night period. The videos were recorded with low light sensitive black and white digital cameras (Square black and white CCD camera, model F60B/N80-50G, KT&C Co. Ltd., Korea Technology and Communications Korea, supplied in waterproof housing by Praesentis S.L. Barcelona, Spain), installed just below the water surface. The cameras were connected to a digital video recorder (model XMOTION-304H supplied by Praesentis, S.L).

2.5.4. Statistical analysis

All data means were presented with standard error of the mean (SEM). The weight and length distributions from the replicated groups after the initial sample months lost a normal distribution as the distributions became positively skewed towards a few large individuals in the sample. The data were compared with the two-way Scheirer-Ray-Hare extension of the Kruskal-Wallis test described in Sokal and Rohlf 1995. Basically the data was ranked and compared with a two-way ANOVA. Weight or length were the dependant variables and RAS system (1, 2 or 3) and treatment feeding system (programmed or auto-demand) were the independent variables. All pairwise multiple comparison were made with the Holm-Sidak method. Differences in growth (weight and length) other time (months) were tested with a Kruskal–Wallis ANOVA test by ranks. Multiple comparison were made with the DUNNS test. The specific growth rate (SGR), coefficient of variation (CV) and feed conversion ratio (FCR) were compared with a three-way ANOVA. The parameters SGR, CV and FCR were the dependant variables and RAS system (1, 2 or 3), treatment feeding system

(programmed or auto-demand) and month of sampling were the independent variables. Pairwise multiple comparisons for CV on different dates were made with the Holm-Sidak method. The weight distributions of the populations were compared using two-sample Kolmogorov-Smirnov test. Cumulative number of demands of feed during the night and day were compared with the 2 way Scheirer-Ray-Hare extension of the Kruskal-Wallis test described above, with cumulative number of demands as the dependant variable and RAS system (1, 2 or 3) and day / night were the independent variables. The swimming velocity of fish was compared using a repeated measures two-way ANOVA. There were no differences in swimming velocity between days. Therefore, in the ANOVA, the tank was the subject that was repeatedly measured, swimming speed was the dependant variable and time of day (08:30, 10:30, 13:30, 16:30 and 18:30) and treatment feeding system (programmed or auto-demand) were the independent variables. All pairwise multiple comparison were made with the Holm-Sidak method. Distributions of the fin condition (index 1, 2, 3 and 4) were compared using the chi square test. The ANOVA analysis were made using Sigma stat 3.5 (Systat Software Inc., USA) statistical package. The Two-sample Kolmogorov-Smirnov test and Chi squared tests were made using excel and the appropriated statistical tables. Statically significant differences were reported when $P<0.05$.

3. RESULTS

3.1 Effect of stimuli in the feeding behavior of meagre

3.1.1 The effect of stimuli on the feeding behavior of meagre with body weight 50-100 g Exp1 and Exp2,

In both Exp1 and Exp2 the light stimulus had a direct effect on the groups. The fish were attracted to the light stimulus when applied during the three daily feeding periods. This was shown from the distance measurements of the fish from the feeding area when compared to periods with and without stimuli (P<0.05) (**Fig. 3.1.1.1a, b, c**).

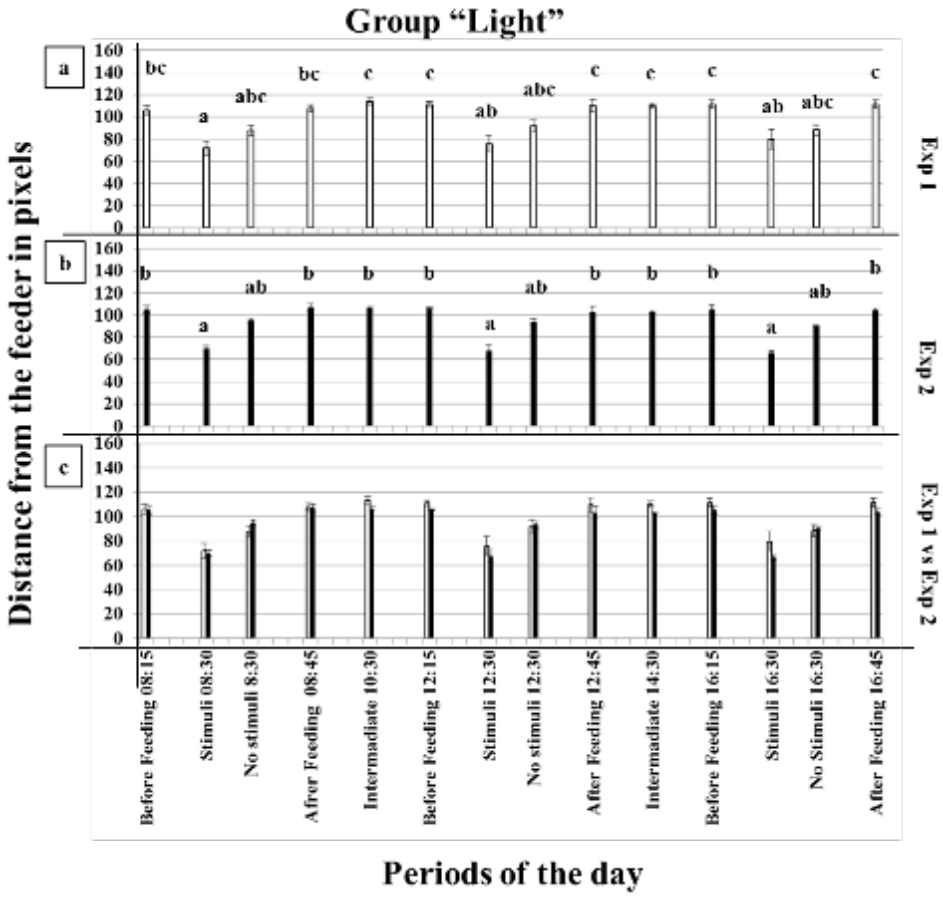

Figure 3.1.1.1. Distance in pixels (mean ± SE) from the feeder of the "Light" group during the different periods of the day of a) Exp1, b) Exp2 and c) both Exp1 and Exp2. Statistically significant differences between means are indicated with different letters above the means (ANOVA, Tukey test, P < 0.05).

In Exp1, in the "Air" group (**Fig. 3.1.1.2a**) the fish were repelled from the feeding area during the application of air stimuli. In Exp2 the experimental population from the "Air" group was not affected by the air stimuli application (**Fig. 3.1.1.2b**). However, the air stimuli had a positive effect to the fish of Exp2 during implementation, as the fish were attracted more to the feeding area than fish of Exp1 (**Fig. 3.1.1.2c**).

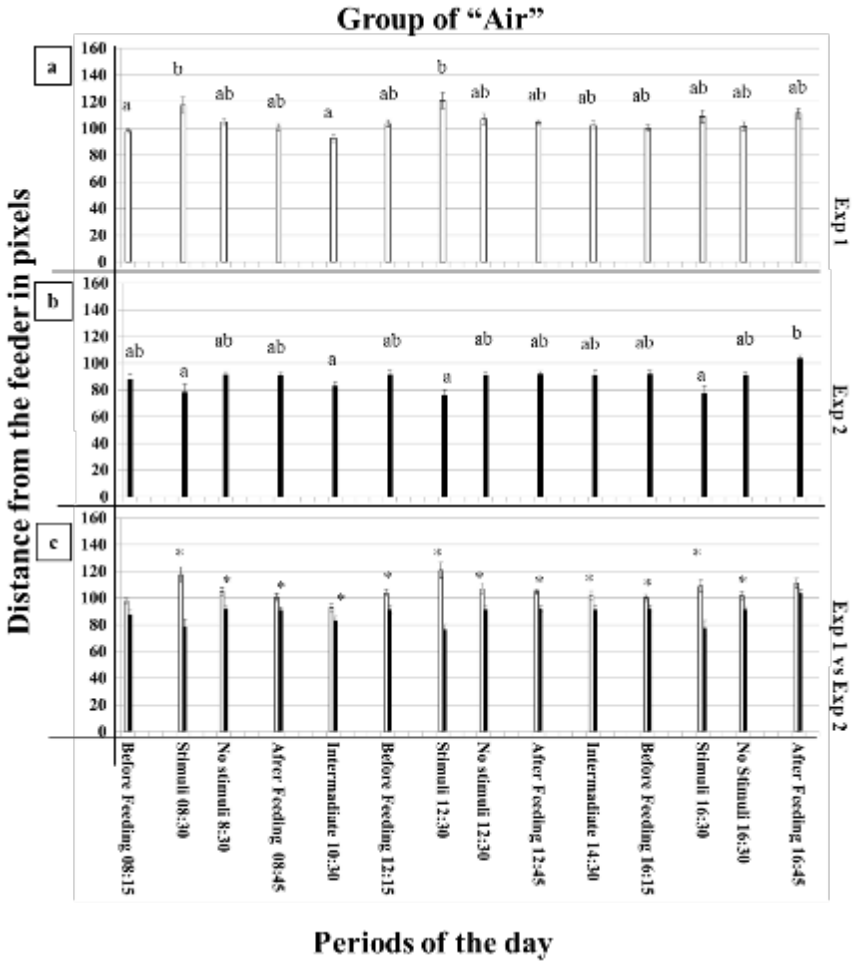

Figure 3.1.1.2. Distance in pixels (mean ± SE) from the feeder of the group with the air stimulus during the different periods of the day of a) Exp1, b) Exp2 and c) both Exp1 and Exp2. Statistically significant differences between means are indicated with different letters above the means (ANOVA, Tukey test, $P < 0.05$). The asterisk indicates statistical differences between Exp1 and Exp2 for each specific period of the day.

In the case of the group that was subjected to the combined stimuli (light in the morning and afternoon and air at noon), the fish did not respond to the stimuli during implementation in Exp1 (**Fig. 3.1.1.3a**). Nevertheless, at Exp2 fish were attracted to the feeding area by the light stimuli, during the morning and the afternoon hours, than by the "Air" stimulus, at noon. Furthermore, even though the "Air" stimulus had a lower attractiveness to the feeding area in comparison to the "Light" stimulus, it had a general effect in attracting the fish to the feeding area in comparison with the other periods of the day (**Fig. 3.1.1.3b**). Furthermore, at Exp2 the experimental population was attracted closer to the feeding area during the stimuli implementation in comparison to Exp1 (**Fig. 3.1.1.3c**).

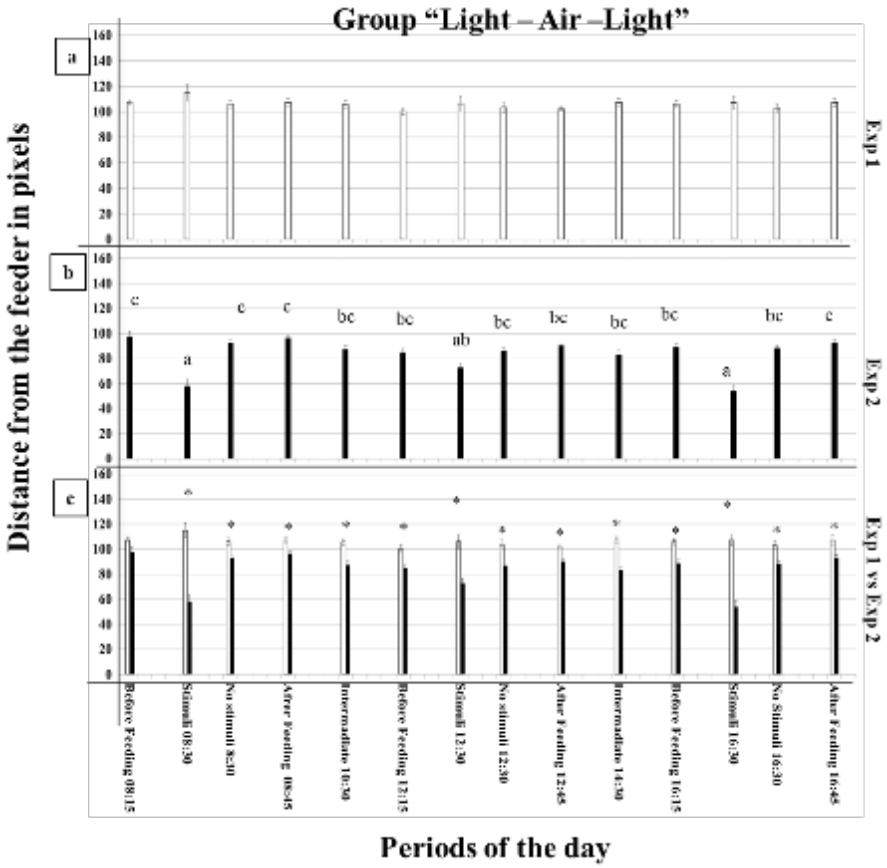

Figure 3.1.1.3. Distance in pixels (mean ± SE) from the feeder of the group with the combined stimuli (light stimulus in the morning and afternoon and air stimulus at noon) during different periods of the day of a) Exp1, b) Exp2 and c) both Exp1 and Exp2. Statistically significant differences between means are indicated with different letters above the means (ANOVA, Tukey test, $P < 0.05$). The asterisk indicates statistical differences between Exp1 and Exp2 for each specific period of the day.

No differences in the position of the experimental population were observed during the different periods of the day at the control group of both experiments **(Fig. 3.1.1.4a, b)**. However, the mean distances from the experimental individuals of Exp2 were detected to be closer to the feeding area during most of the different periods of the day **(Fig. 3.1.1.4c)** in comparison with the distances of experimental population that were detected at the Exp1 ($P < 0.05$).

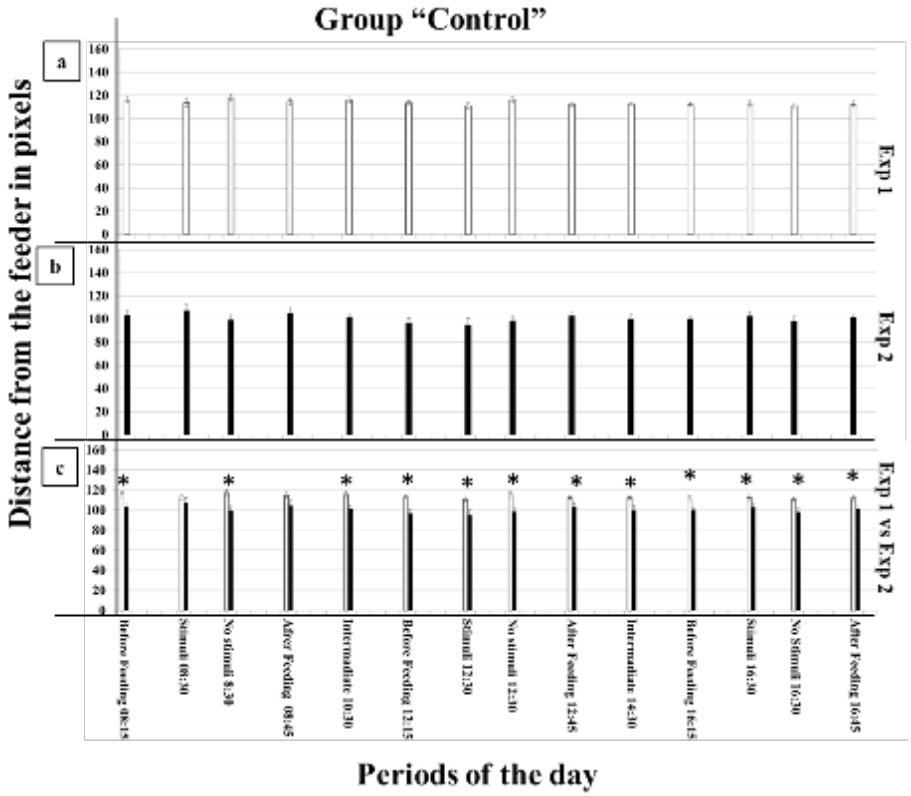

Figure 3.1.1.4. Distance in pixels (mean ± SE) from the feeder for the control group in different periods during the day of a) Exp1, b) Exp2 and c) both Exp1 and Exp2. The asterisk indicates statistical differences between Exp1 and Exp2 for each specific period of the day (ANOVA, Tukey test, $P < 0.05$).

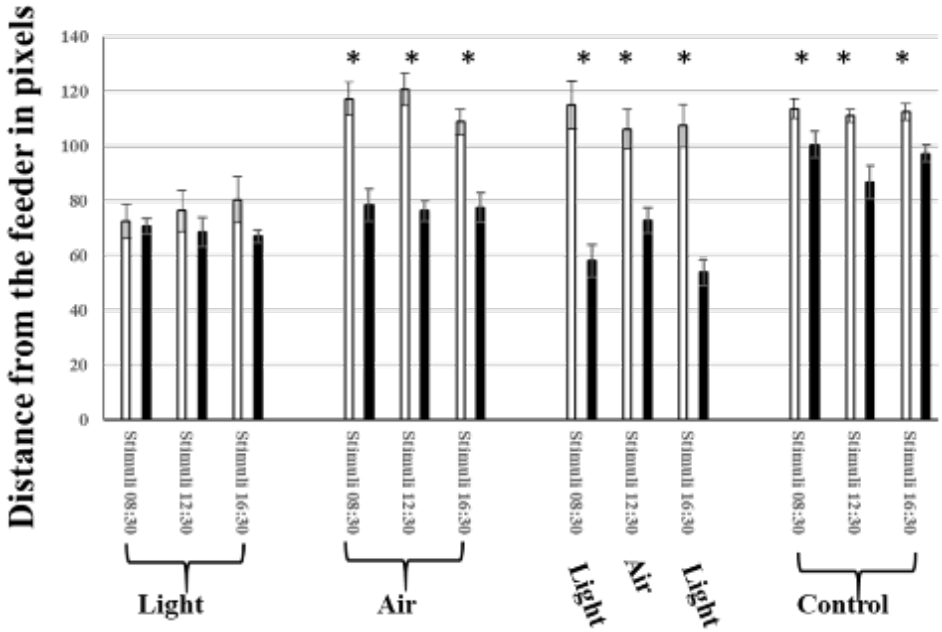

Figure 3.1.1.5. Distance of fish from the feeder during the different periods of the day for different stimuli applications, of Exp1 (white column) and Exp 2 (black column) (values are mean ± SE). Asterisks (*) indicate differences between experiments (Two Way ANOVA, Tukey test, $P<0.05$).

Concerning the comparison between Exp1 and Exp2, statistical differences ($P < 0.05$) appeared in all of the groups except the "Light" group **(Fig. 3.1.1.5)**, which means that the experimental populations were attracted by the light stimuli at the same degree in both experiments Exp1 and Exp2. It is remarkable that there were statistical differences in the control group between Exp1 and Exp2, as the distance from the feeder during the period of stimuli application in the other groups the population at the control group was closer to the feeder at Exp2 in comparison to Exp1 ($P < 0.05$).

3.1.2. The effect of stimuli on the feeding behavior of meagre with body weight 600-900 g.

During the morning and in the afternoon the fish were distributed in most areas of the tank (**Fig. 3.1.2.1a, b**). At noon the experimental population remained in the shaded area of the tank (**Fig. 3.1.2.1c**). During the morning and afternoon hours of stimuli implementation the fish were attracted to the feeding area by both air and light stimuli (**Fig. 3.1.2.1e, f**). On the contrary, during noon the air stimulus attracted more than the others experimental groups (which previously preferred to remain at the dusky area) at the feeding area (**Fig. 3.1.2.1g**). This was more intense compared to light stimuli, (even though the feeding area was at the light part of the tank). Concerning the effect of the different stimuli that were performed, the one of air bubbles had a direct, positive effect on the population. This is because it was shown that during its implementation, the fish were attracted to the feeding area during all the feeding periods of the day.

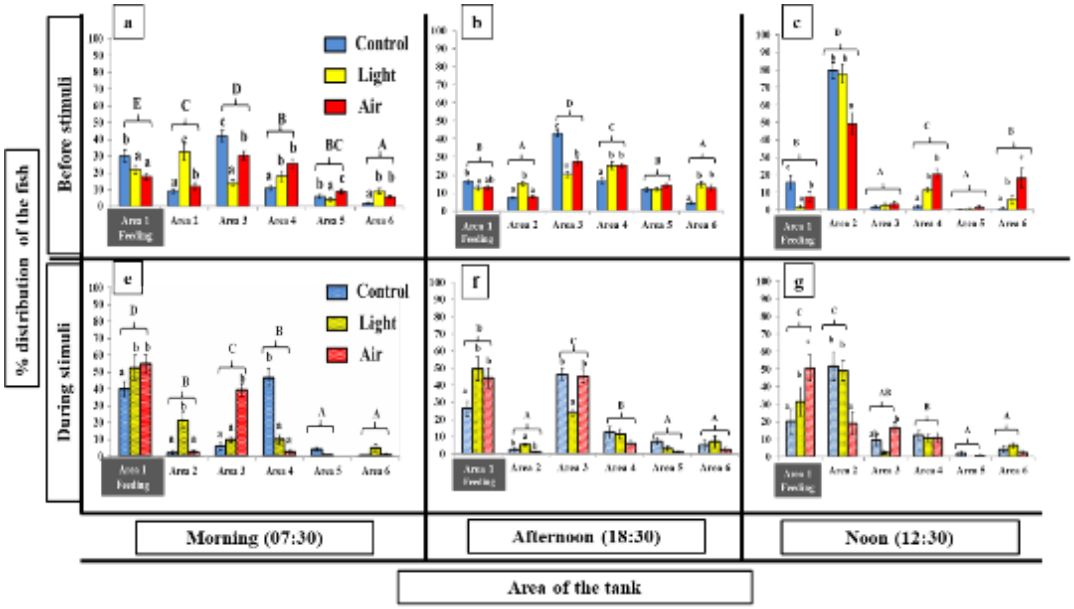

Figure 4. The distribution (%) of the fish in the different areas of the tanks during different periods of the day. The graphs present the periods before (a, b, c) and during stimuli application (e, f, g). Values are mean ± SE. Latin characters with uppercase letters indicate differences between the different areas and with lowercase letters differences between the conditions for each area (Two Way ANOVA, Duncan test, $P<0.05$).

Attraction of the fish population to the feeding area by the air stimulus was acquired from the 2nd day of application, but this observation was more stable after the 5th and 7th experimental days. Additionally, the attraction by the air stimulus was clearly visible during all the periods of the day that it was activated. On the contrary, the fish groups to which the light stimulus was applied, responded to its application with 6 days delay, between the 12th-14th experimental day and the fish

were attracted to the feeding area only at the morning and afternoon feeding times. At noon, the light stimulus overlapped with the high sunlight intensity and the stimulus became invisible to the fish.

An important observation in this experiment was the effect of weather conditions and especially of the sunlight intensity on meagre feeding behavior. The constant daily movement of sun during the day had a large impact on the different parts of the experimental tanks (**Fig. 3.1.2.2a, b, c, d, e**). It created differentiations in the light intensity within the tank environment when the tank was exposed to the direct sunlight. There were shaded and light areas in the tank. During sunny days the fish in all replicates preferred to stay in the shaded than in the light area of the tank. Especially whilst feeding at noon (12:30 am), the above observation was very intense during sunny days while during cloudy days the fish presented a similar distribution pattern as during morning or afternoon.

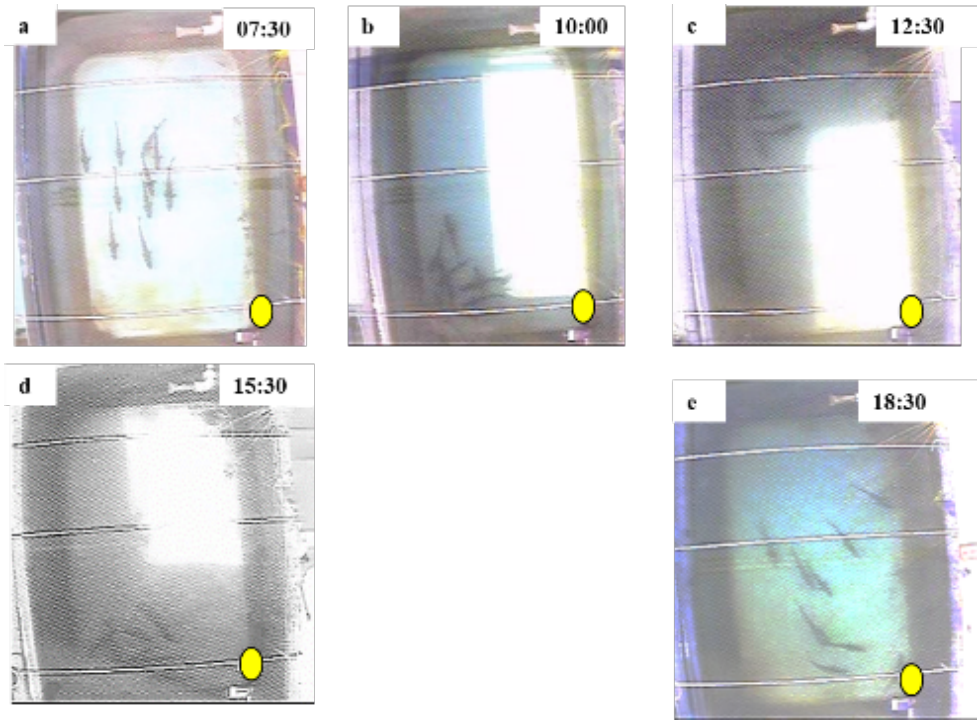

Figure 3.1.2.2. The distribution of the fish in the different areas of the tank during the different periods of the day (a, b, c, d, e).

3.1.3. Eye histology

The relative cell densities in the different layers of the retina are considered as a comparative indicator for the classification of a species as nocturnal or diurnal. The high number of rods that eventually cover the whole surface of the retina in meagre indicate that the cells in the inner nuclear layer (INL) are able to capture data from an increasing number of rods (**Fig. 3.1.3.1a**). The arrangement of double and single cones on the retina surface follows a specific pattern, which is clearly visible. Pairs of double cones are placed in series (**Fig. 3.1.3.1b**). Additionally, the number of rods, the number of cells in the INL and the ratio between the different types of retina cells are used an indicators in order to clasify a species as nocturnal or diurnal **(Table 3.1.3.1)**.

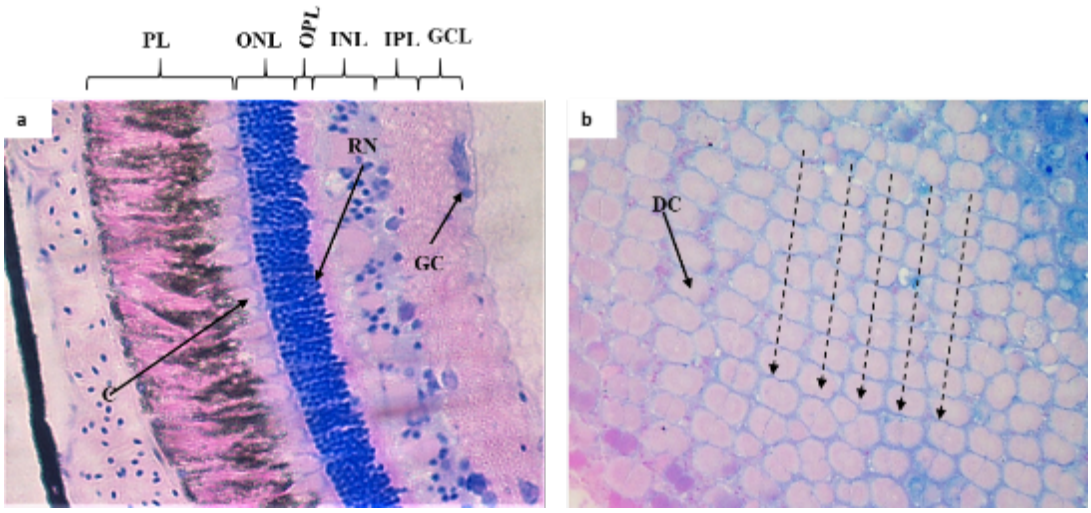

Figure 5. Microphotographs of histological sections of the eye of meagre of a mean weight of 200 ± 20 g, (a) longitudinal section, (b) transverse section at the surface of the retina. The five dashed arrowhead lines are placed on the common contact surface of the members single cone that constitutes the pair of double cones. GCL = ganglia cell layer, IPL = inner plexiform layer, INL = inner nuclear layer, OPL = outer plexiform layer, ONL = outer nuclear layer, PL = photoreceptor layer, GC = ganglia cells, RN = rod nucleus, C = cones, DC= double cone.

Table 3.1.3.1. Values (means and standard deviations) of the different structures that the retina consists of in 100-µm length areas of histological sections of the eye of meagre, such, as cones, rods, cells in the inner nuclear layer, ganglia cells and the ratios between them. The size of meagre was 200 ± 20 g (n = 6).

	Mean	Standar deviation
Rods	164.03	19.75
Cones	6.25	0.99
INL	35.53	4.97
Ganglia	4.72	1.10
Cones/Rods	0.04	0.00
Ganglia/INL	0.14	0.04
Rods/Ganglia	37.27	14.00
Rods/ INL	4.68	0.73
Cones/Ganglia	1.44	0.67

3.2 Test of different feeding methods

3.2.1 Growth and feeding performance.

EXP1 Individual size of 50-100 g

All feeding methodologies tested during EXP1 provided satisfactory growth results. Moreover, the daily feed consumption (DFC) was higher ($P<0.05$) when using the hand feeding method in comparison with the other methods. However, these differences did not significantly reflect on the FCR values between the different feeding methodologies that were used (**Table 3.2.1.1**).

Table 3.2.1.1. Evolution of productive indexes during EXP1. Latin characters indicate differences between the different feeding methods (One Way ANOVA, Duncan test, $P<0.05$).

	Self feeder	Hand feeding	Automatic feeder
Weight (initial)	64.71 ± 1.96	62.23 ± 2.90	64.58 ± 0,79
Weight (final)	132.48 ± 11.60	133.59 ± 5.22	138.24 ± 1.63
SGR	0.80 ± 0,08	0.86 ± 0,02	0.86 ± 0,01
FCR	1.07 ± 0.11	1.25 ± 0.10	1.15 ± 0.03
DFC	0.73 ± 0.07^{a}	0.92 ± 0.07^{b}	0.84 ± 0.01^{a}
CF (initial)	0.99 ± 0.04	1.02 ± 0.02	0.96 ± 0.01
CF (final)	0.92 ± 0.04	0.93 ± 0.02	0.94 ± 0.02

EXP2. Individual size of 700-900 g

Experimental groups fed either by hand or with the automatic feeders showed the highest SGR in comparison with the ones fed with self-feeders ($P<0.05$). Similarly, statistically significant differences appeared in the DFC, where lower feeding rates were presented in the self-feeder group ($P<0.05$). Although there were no statistical significant differences between the feeding methodologies for the FCR, higher values were obtained with the automatic feeder (**Table 3.2.1.2**).

Table 3.2.1.2. Evolution of productive indexes during EXP2. Latin characters indicate differences between the different feeding methods (One Way ANOVA, Duncan test, $P<0.05$).

	Self feeder	Hand feeding	Automatic feeder
Weight (initial)	739.88 ± 23.38	775.95 ± 57.39	673.04 ± 79.35
Weight (final)	927.72 ± 4.50	1090.00 ± 95.14	905.90 ± 112.49
SGR	0.32 ± 0.05^{a}	$0.49 \pm 0,02^{b}$	0.42 ± 0.01^{ab}
FCR	1.27 ± 0.27	1.42 ± 0.23	1.83 ± 0.19
DFC	0.40 ± 0.02^{a}	0.68 ± 0.09^{b}	0.77 ± 0.06^{b}
CF (initial)	0.95 ± 0.00	0.94 ± 0.00	0.93 ± 0.04
CF (final)	1.10 ± 0.02	1.06 ± 0.07	1.02 ± 0.00

3.2.2 Distribution of the fish

In the indoor tanks, (EXP 1) where the light intensity was low, the distribution of the fish was homogenous during the period of the day (**Fig. 3.2.2.1**). On the contrary in the outdoor tanks (EXP 2) the distribution of the fish in the morning and the afternoon was homogenous, but under direct sunlight fish remained at the shaded area of the tanks (**Fig. 3.2.2.1**).

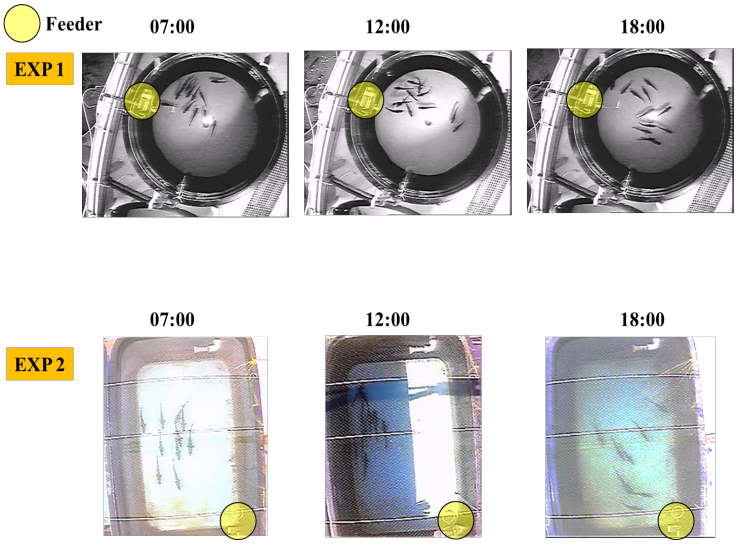

Figure 3.2.2.1. Distribution of the fish during the different periods of the day. The transparent yellow cycle area indicates the place of the feeder in the case of the self-feeder and the automatic feeder.

3.2.3 Feeding activity

The data from the self-feeder recorder, was analyzed. The feeding activity of the fish in both experiments was statistically significantly lower during the morning and until noon, rather than during the rest of the day. More specifically in both experiments (EXP1 and EXP2) between 06:00 and 12:00 the fish had a lower feeding activity, while this activity had increased during the remaining of the period (**Fig. 3.2.3.1a, b**). This difference was more intense in the outdoor tanks, where fish were exposed to direct sunlight and the intensity of light was much higher.

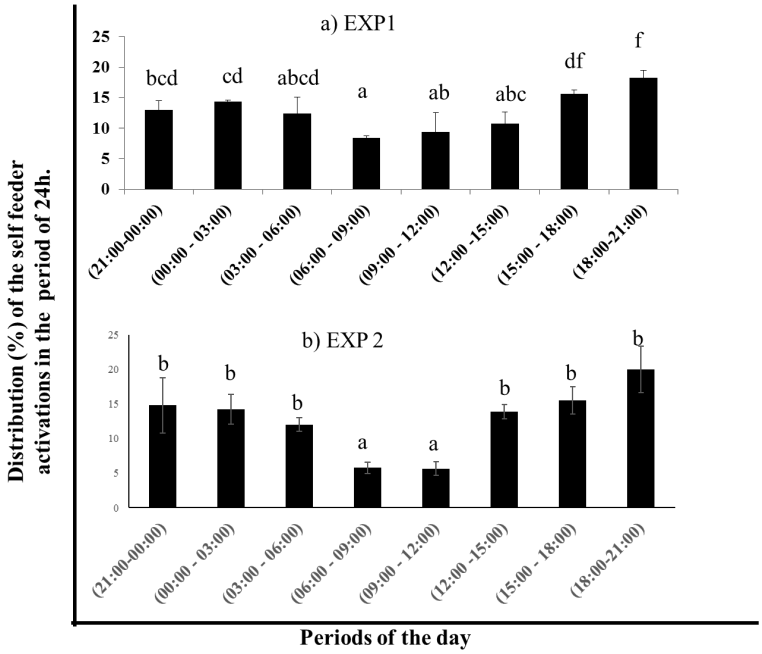

Figure 3.2.3.6. The self-feeder activity (as percentage of the total daily) during the 24h period for (a) EXP1 and (b) EXP2. Different letters indicate statistical differences (Two Way ANOVA, Student – Newman – Keuls Method, $P<0.05$).

3.2.4 Evacuation rates of the digestive canal

The results of the evacuation rates of the digestive canal, showed that at 19 °C, the stomach is completely empty after 24 hours (**Fig. 3.2.4.1**). During the first 8 hours, the stomach lost 50% of its content. After that, the evacuation rate is relatively slower. Contrary to this, the intestine reaches its maximum filling 12 hours from the first feeding, but within 24 hours negligible food residues were identified in the intestine.

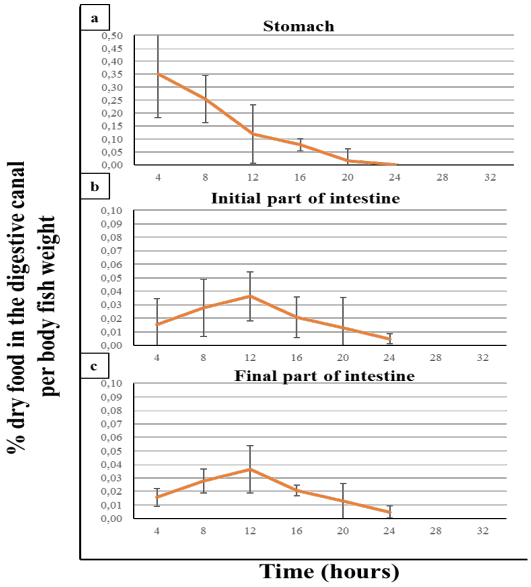

Figure 3.2.4.1. Evacuation rates of a) stomach, b) initial part of intestine and c) final part of intestine.

3.3 Test of daily vs night feeding in the performance of meagre

3.3.1 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.
In **Figure 3.3.1.1** the growth performance is presented.

Figure 3.3.1.1 Growth performance, mean weight, of meagre. Error bars are the standard deviation (n=10)

During the period the growth rate was ~2.5 g d^{-1} with no significant difference observed between the tested conditions. The final weight presented a coefficient of variation for all groups between 21.3 and 29.2% with the night-fed groups being the ones with the higher variation. The size

distribution at the end of the trial was canonical (**Figure 3.3.1.2**) for all groups although a tendency towards lower sizes was observed for the night-fed groups.

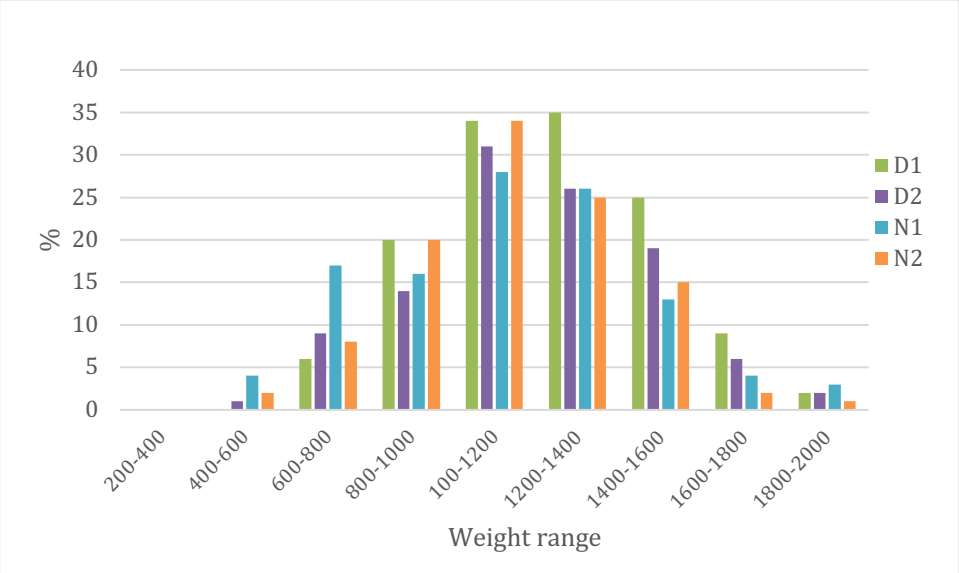

Figure 3.3.1.2 Size distribution at the end of the trial

Regarding other performance indicators, in **Table 3.3.1.1** the mortality (as %) and the food conversion ratio are presented. No significant differences were observed between groups.

Table 3.3.1.1 Performance indicators during the two experimental phases

	D1	D2	N1	N2
Mortality (%)	4.8	3.5	5.4	2.6
FCR	2.6	2.6	3.0	2.7

3.3.2 Hematological, Biochemical and Hormonal parameters

Hematocrit

Hematocrit levels appear to be affected both by the sampling period ($P < 0.001$) and the condition (feeding during night or day) with a statistically significant interaction between the two factors (**Figure 3.3.2.1**). During MAY and OCTOBER hematocrit levels appeared statistically higher ($P < 0.05$) in the fish that were fed during day (MAYday = 34.0 ± 5.2 %; OCTday = 37.1 ± 4.9 %) compared to those that were fed during night (MAYnight = 30.4 ± 2.9 % OCTnight = 31.1 ± 3.5 %). For both groups of fish the lowest hematocrit levels were observed during JULY (JULday = 28.6 ± 4.9 %; JULnight = 29.1 ± 2.0 %). For the group that was fed during day the highest levels were observed in OCTOBER (OCTday = 37.1 ± 4.9 %) whereas for the other group in DECEMBER (DECnight = 35.3 ± 3.3 %).

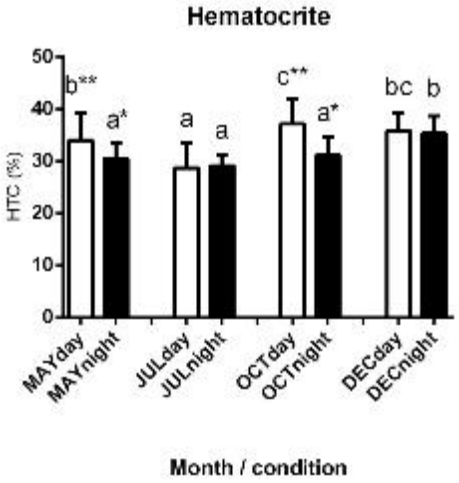

Fig 3.3.2.1. Hematocrit levels during the period from May 2016 to December 2016 (May/day feeding: MAYday; May/night feeding: MAYnight; July/day feeding: JULday; July/night feeding: JULnight; October/day feeding: OCTday; October/night feeding: OCTnight; December/day feeding: DECday; December/night feeding: DECnight. Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months) and asterisks differences between the different feeding schemes, $P < 0.05$.

Hemoglobin

Hemoglobin levels appear to be affected both by the sampling period ($P < 0.001$) and the treatment (feeding during night or day) only during OCTOBER. During this sampling period the highest hemoglobin levels were observed and also the levels in the fish that were fed during day were statistically higher compared to the night group (OCTday = 6.9 ± 1.6 mmol L^{-1}; OCTnight = 6.1 ± 2.1 mmol L^{-1}) with no statistically significant interaction between the two factors (**Figure 3.3.2.2**).

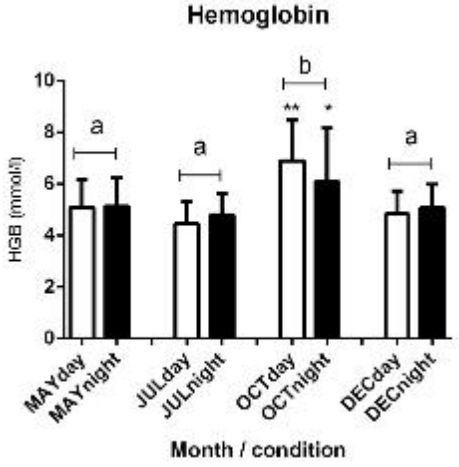

Fig 3.3.2.2. Hemoglobin levels during the period from May 2016 to December 2016 (May/day feeding: MAYday; May/night feeding: MAYnight; July/day feeding: JULday; July/night feeding: JULnight; October/day feeding: OCTday; October/night feeding: OCTnight; December/day feeding: DECday; December/night feeding: DECnight. Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months) and asterisks differences between the different feeding schemes, $P < 0.05$.

Glucose

Glucose levels were affected both by the sampling period and the feeding schemes ($P < 0.001$) with highest levels observed in JULY (JULday = 103.7 ± 35.8 mmol L^{-1}; JULnight = 108.1 ± 35.3 mmol L^{-1}) (**Figure 3.3.2.3**). The lowest glucose levels were observed during MAY when an effect of the feeding scheme ($P < 0.05$) was also observed showing lower lever in fish fed during night compared to the other group (MAYday = 56.0 ± 37.5 mmol L^{-1} ; MAYnight = 89.2 ± 38.5 mmol L^{-1}).

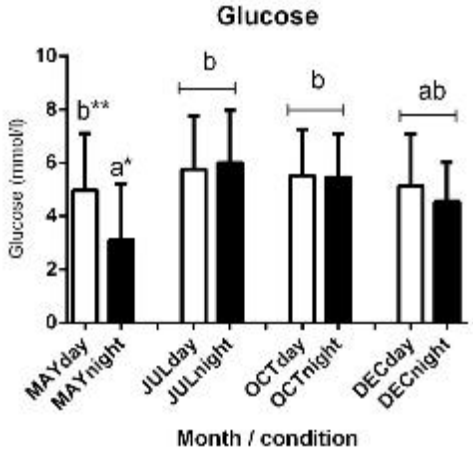

Fig 3.3.2.3. Glucose levels during the period from May 2016 to December 2016 (May/day feeding: MAYday; May/night feeding: MAYnight; July/day feeding: JULday; July/night feeding: JULnight; October/day feeding: OCTday; October/night feeding: OCTnight; December/day feeding: DECday; December/night feeding: DECnight. Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months) and asterisks differences between the different feeding schemes, $P < 0.05$.

Lactate

No effect either of the sampling period or the feeding scheme were observed in the case of plasma lactate levels as depicted in **Figure 3.3.2.4**.

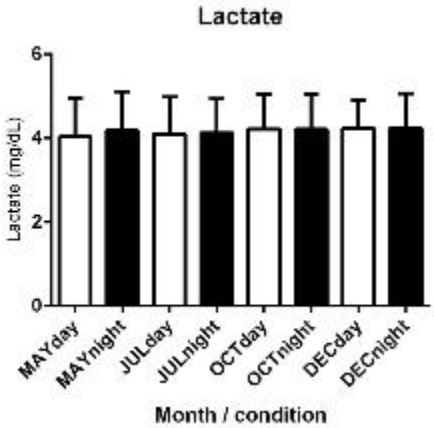

Fig 3.3.2.4. Lactate levels during the period from May 2016 to December 2016 (May/day feeding: MAYday; May/night feeding: MAYnight; July/day feeding: JULday; July/night feeding: JULnight; October/day feeding: OCTday; October/night feeding: OCTnight; December/day feeding: DECday; December/night feeding: DECnight. Values are given as mean ± S.D. (n = 10 per group and sampling month).

Cortisol

No effect either of the sampling period or the feeding scheme were observed in the case of plasma cortisol levels as depicted in **Figure 3.3.2.5**.

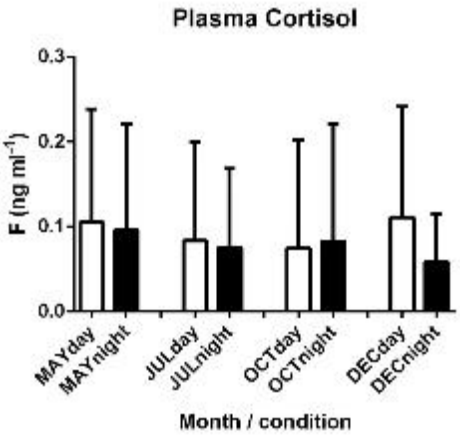

Fig 3.3.2.5. Plasma cortisol levels during the period from May 2016 to December 2016 (May/day feeding: MAYday; May/night feeding: MAYnight; July/day feeding: JULday; July/night feeding: JULnight; October/day feeding: OCTday; October/night feeding: OCTnight; December/day feeding: DECday; December/night feeding: DECnight. Values are given as mean ± S.D. (n = 10 per group and sampling month).

3.3.3 Behavioral monitoring

All data collected were analyzed in terms of difference between the tested conditions. Significant alteration was observed between the experimental groups. The groups in the day-fed cages expressed the expected behavior during the day light with the majority of the individuals concentrated at the lower layers of the cage and with observed movements towards the surface when feeding occurred. In the echograms the feeding periods are clearly marked with the vertical movements of the fish towards feeds, followed by a return to the lower layers of the cage.

During night individuals were almost homogenously distributed in the cage. The groups in the night-fed cages presented two distinct behaviors during the trial. At the beginning and for a period of 2-3 months the fish expressed an anticipatory behavior searching for feed during the day although they were fed during the night period. It was only after this period that the fish were calm during the day time and were fed normally during night (**Figure 3.3.3.1**).

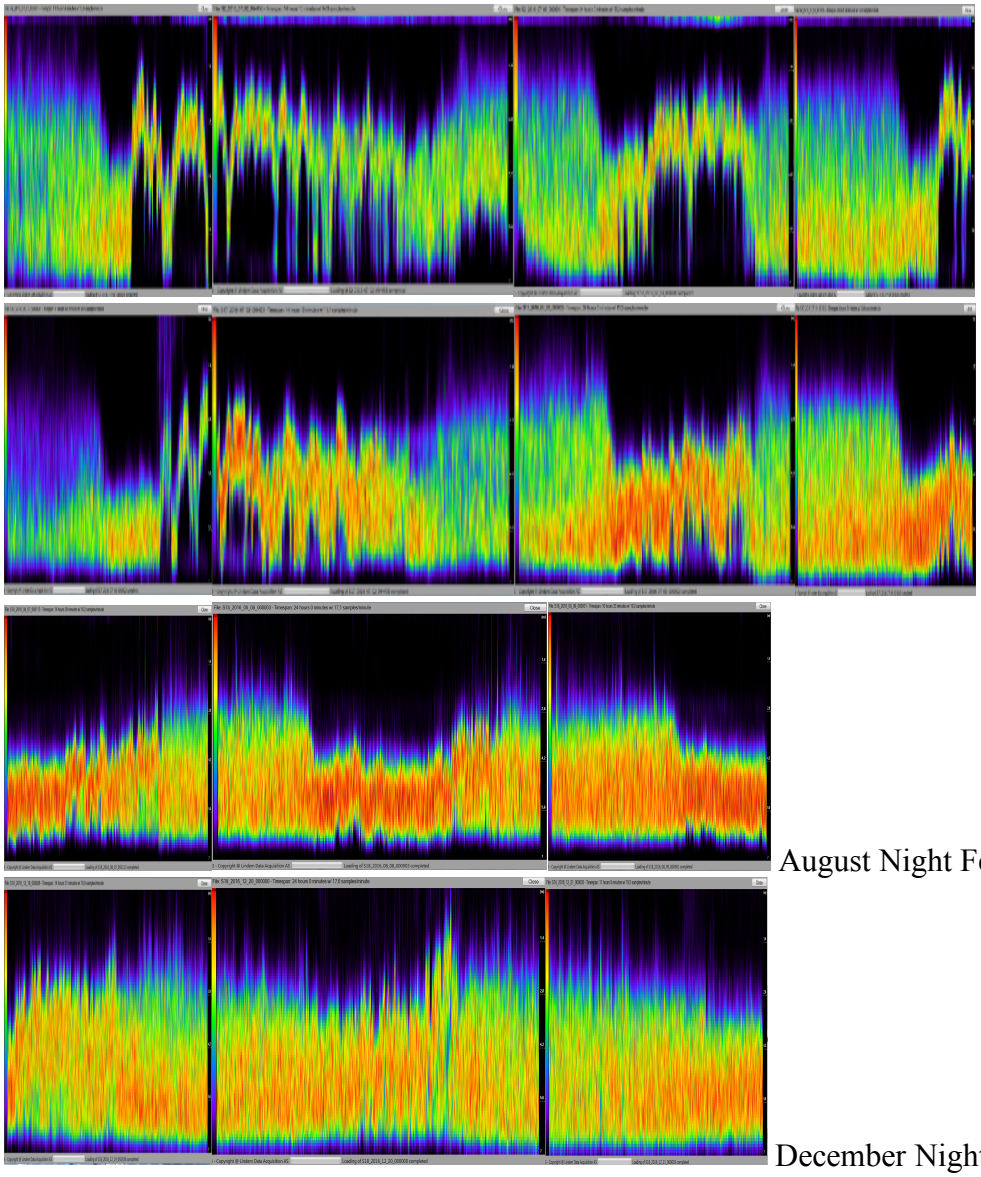

Figure 3.3.3.1. Vertical distribution of meagre in the experimental cages during the trial. Horizontal Axis is the time while Vertical is the cage depth (from bottom to top). Different colors (from violet to red) represent different densities.

In Appendix 1 the results of all observations during the experiment are presented. The observations were made for periods between 2 up to 7 days. Also, different conditions relative to weather, currents, human presence, feeding were observed. This behavior is in general different from what was observed in salmon (Oppedal et al 2011) or European seabass (Papandroulakis et al 2012) cage farming 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 anticipatory behavior may express the capacity of the individuals to feed during the whole day period. This was tested during the trial described in the 5th experiment of the present deliverable.

3.4 Comparing two feed distribution methods in the cages

3.4.1 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.
In **Figure 3.4.1.1** the growth performance is presented.

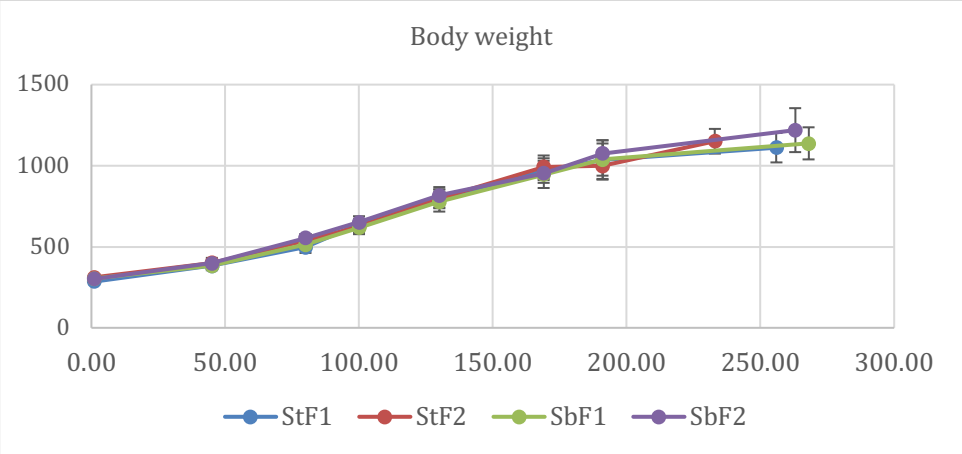

Figure 3.4.1.1 Growth performance, mean weight, of meagre. Error bars are the standard deviation (n=10)

During the period the growth rate was ~3.8 g d^{-1} for all conditions tested with no significant difference was observed between or within the tested conditions. The final weight presented a coefficient of variation for all groups between 20.6 and 25.7% without differences between the tested conditions. The size distribution at the end of the trial was canonical (**Figure 3.4.1.2**) for all groups although a tendency towards bigger sizes was observed for the submerged fed groups.

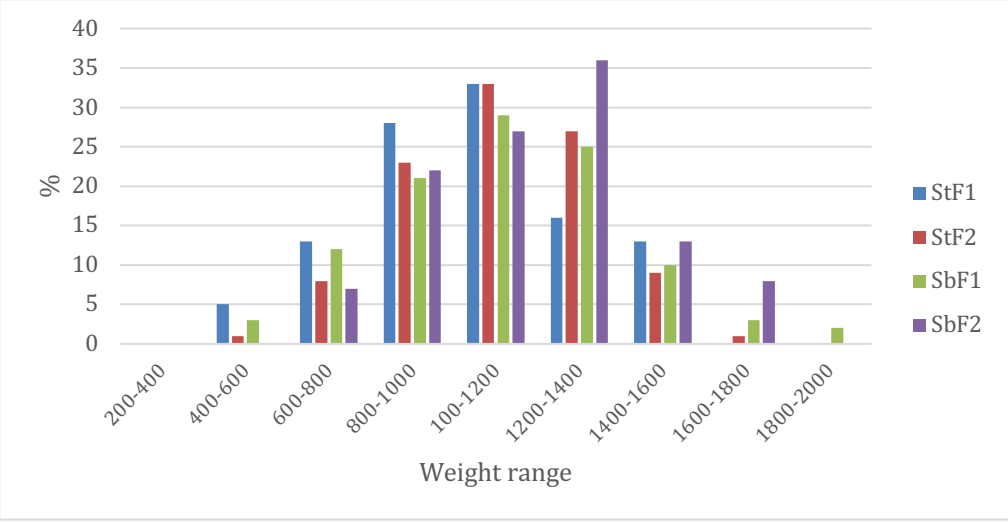

Figure 3.4.1.2 Size distribution at the end of the trial

Regarding other performance indicators, in Table 3.4.1.1 the mortality (as %) and the food conversion ratio are presented. No significant differences were observed between groups.

Table 3.4.1.1 Performance indicators during the two experimental phases

	StF1	StF2	SbF1	SbF2
Mortality (%)	3.2	3.8	3.4	2.3
FCR	1.7	1.5	1.8	1.6

3.4.2 Hematological, Biochemical and Hormonal parameters

Hematocrit

Hematocrit levels appear not to be affected either by the sampling period or the treatment (surface feeding vs submerged feeding) with values ranging from 26 to 48% (**Figure 3.4.2.1**).

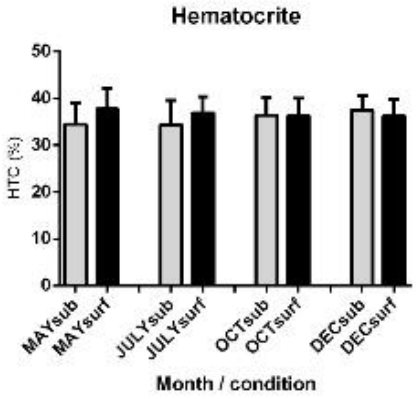

Fig 3.4.2.1. Hematocrit levels during the period from May 2017 to December 2017 (May/ submerged feeding: MAYsub; May/ surface feeding: MAYsurf; July/ submerged feeding: JULsub; July/ surface feeding: JULsurf; October/ submerged feeding: OCTsub; October/ surface feeding: OCTsurf; December/ submerged feeding: DECsub; December/ surface feeding: DECsurf. Values are given as mean ± S.D. (n = 10 per group and sampling month).

Hemoglobin

Hemoglobin levels appear not to be affected either by the sampling period or the treatment (surface feeding vs submerged feeding) with values ranging from 1.88 to 4.8 mmol L^{-1}(**Figure 3.4.2.2**).

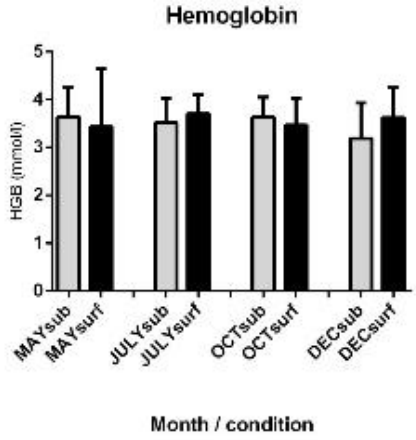

Fig 3.4.2.2. Hemoglobin levels during the period from May 2017 to December 2017 (May/ submerged feeding: MAYsub; May/ surface feeding: MAYsurf; July/ submerged feeding: JULsub; July/ surface feeding: JULsurf; October/ submerged feeding: OCTsub; October/ surface feeding: OCTsurf; December/ submerged feeding: DECsub; December/ surface feeding: DECsurf. Values are given as mean ± S.D. (n = 10 per group and sampling month).

Glucose

Glucose levels (**Figure 3.4.2.3)** were affected both by the sampling period and the feeding schemes ($P < 0.001$) with a statistically significant interaction between month and condition. For both groups the lowest levels were observed during DECEMBER (DECsub = 1.09 ± 1.12 mmol L^{-1}; DECsurf = 1.25 ± 0.94 mmol L^{-1}). Highest levels observed in MAY when (MAYsub = 4.75 ± 2.15 mmol L^{-1}; MAYsurf = 4.88 ± 1.98 mmol L^{-1}). During OCTOBER an effect of the feeding schemes was also observed with fish fed using the surface feeding scheme showing higher glucose levels (MAYsub = 4.78 ± 1.21 mmol L^{-1}) than the other group (MAYsurf = 2.98 ± 1.26 mmol L^{-1}).

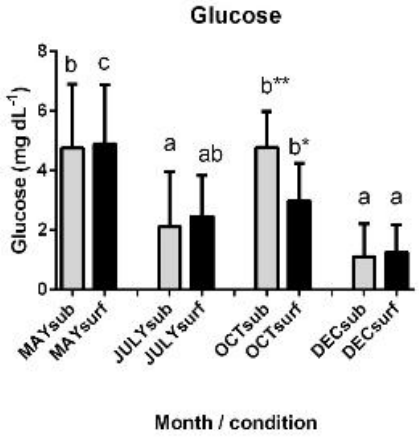

Fig 3.4.2.3. Glucose levels during the period from May 2017 to December 2017 (May/ submerged feeding: MAYsub; May/ surface feeding: MAYsurf; July/ submerged feeding: JULsub; July/ surface feeding: JULsurf; October/ submerged feeding: OCTsub; October/ surface feeding: OCTsurf; December/ submerged feeding: DECsub; December/ surface feeding: DECsurf. Values are given as mean ± S.D. (n = 10 per group and sampling month). Letters indicate differences between the different samplings (months) and asterisks differences between the different feeding schemes, $P < 0.05$.

Lactate

The plasma lactate levels appeared to be affected only by the sampling period ($P < 0.05$) and no effect of the feeding scheme was observed (**Figure 3.4.2.4**).

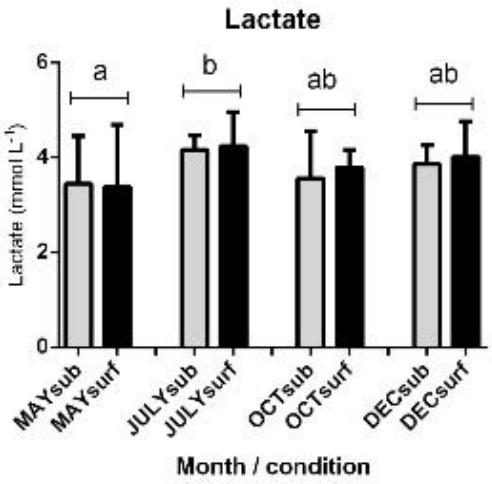

Fig 3.4.2.4. Lactate levels during the period from May 2017 to December 2017 (May/ submerged feeding: MAYsub; May/ surface feeding: MAYsurf; July/ submerged feeding: JULsub; July/ surface feeding: JULsurf; October/ submerged feeding: OCTsub; October/ surface feeding: OCTsurf; December/ submerged feeding: DECsub; December/ surface feeding: DECsurf. 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

No effect either of the sampling period or the feeding scheme were observed in the case of plasma cortisol levels as depicted in **Figure 3.4.2.5**.

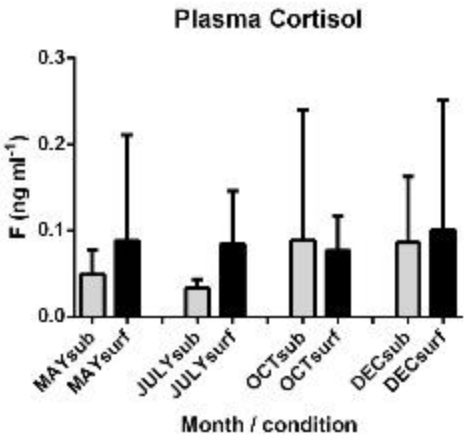

Fig 3.4.2.5. Plasma cortisol levels during the period from May 2017 to December 2017 (May/ submerged feeding: MAYsub; May/ surface feeding: MAYsurf; July/ submerged feeding: JULsub; July/ surface feeding: JULsurf; October/ submerged feeding: OCTsub; October/ surface feeding: OCTsurf; December/ submerged feeding: DECsub; December/ surface feeding: DECsurf. Values are given as mean ± S.D. (n = 10 per group and sampling month).

3.4.3 Immunological analysis

There was no ceruloplasmin in the samples (below detection limit).
Lysozyme activity (**Figure 3.4.3.1**) was significantly higher from July to November compared to serum samples taken in May 2017 but there were no significant difference between the feeding type (surface or submerged). As shown in deliverable 20.2.1, the summer increase and autumnal peak of lysozyme activity was also apparent in the case of large fish samples especially in deep cages. The difference between sampling times observed here were more intense in fish fed at the surface than in fish fed from the submerged tube.

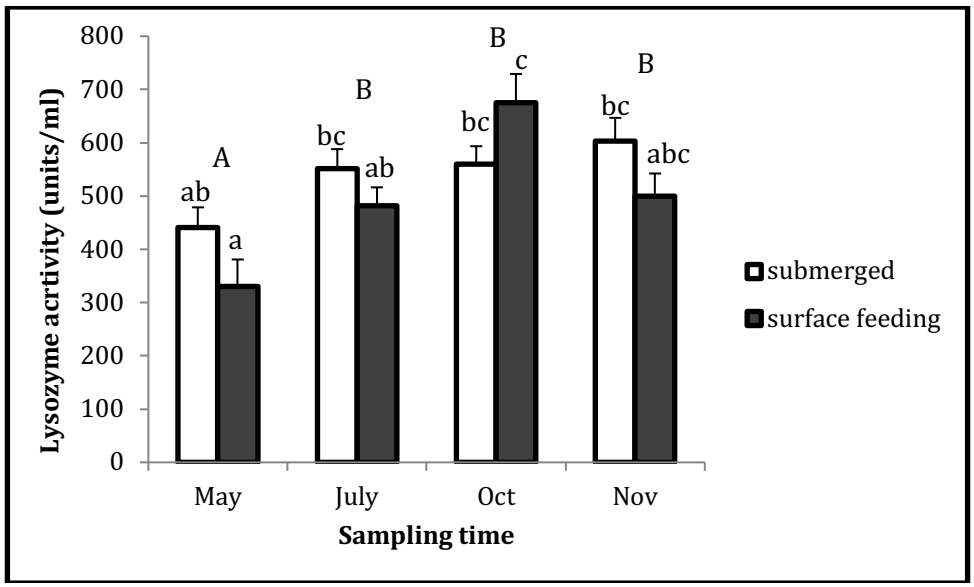

Fig. 3.4.3.1: Lysozyme activity in fish serum sampled at different periods from 4 sea cages. Fish in 2 cages were fed from the surface (open bars) while fish from the 2 other cages were fed from beneath the surface (black bars; submerged feeding). Different uppercase letters show significant differences between sampling periods (General Linear Method, P= 2.10^{-6}). There were no significant difference between feeding types (GLM, P=0.160). Different lowercase letters show significant differences between sampling x feeding types (General Linear Method, P=0.027, One-Way ANOVA P=2.10^{-6}, Tukey t-test). n=20.

Similarly to lysozyme activity, myeloperoxidase activity (**Figure 3.4.3.2**) increased significantly from May to July and even more in October and November. The opposite was true of myeloperoxidase activity of fish sampled for the program Diversify where summer samples showed much higher activity than autumn samples in small fish. The same was true of larger fish but with a slighter reduction of activity when water temperature reduced (Deliverable 20.2). Interestingly in the present study, in July, myeloperoxidase activity of fish fed at the surface was significantly higher than that of fish fed from 10m below the surface. Submerged feeding may retard the summer increase of Myeloperoxidase activity.
Trypsin inhibition (**Figure 3.4.3.3**) also increased significantly from May-July to October-November and fish fed at the surface showed significantly lower trypsin inhibition that submerged –fed fish. Trypsin inhibition was much stronger than that found in fish tested in deep and surface cages but as suggested in Deliverable 20.2, it was stronger in larger fish possibly linked to the higher parasite occurrence in this phase compared to smaller fish.

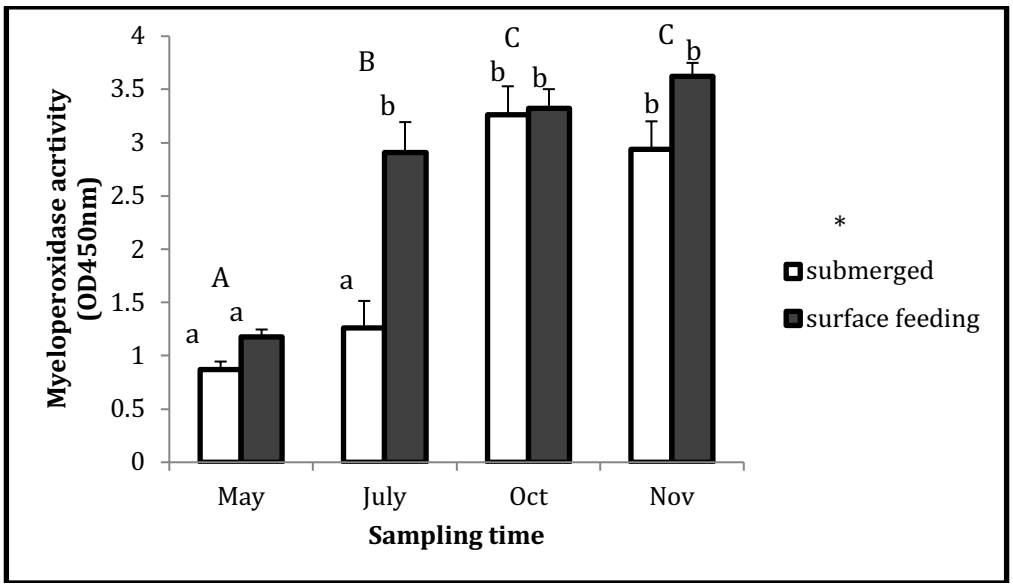

Fig. 3.4.3.2: Myeloperoxidase activity in fish serum sampled at different periods from 4 sea cages. Fish in 2 cages were fed from the surface (open bars) while fish from the 2 other cages were fed from beneath the surface (black bars; submerged feeding). Different uppercase letters show significant differences between sampling periods (General Linear Method, P= 3.10^{-24}). Asterisk show that there was a significant difference between the 2 feeding types (GLM, P=8.10^{-6}). Different lowercase letters show significant differences between sampling x feeding time (GLM, P=0.0009; Kruskal-Wallis, P=6.10^{-6}, tamhane t-test). n=20.

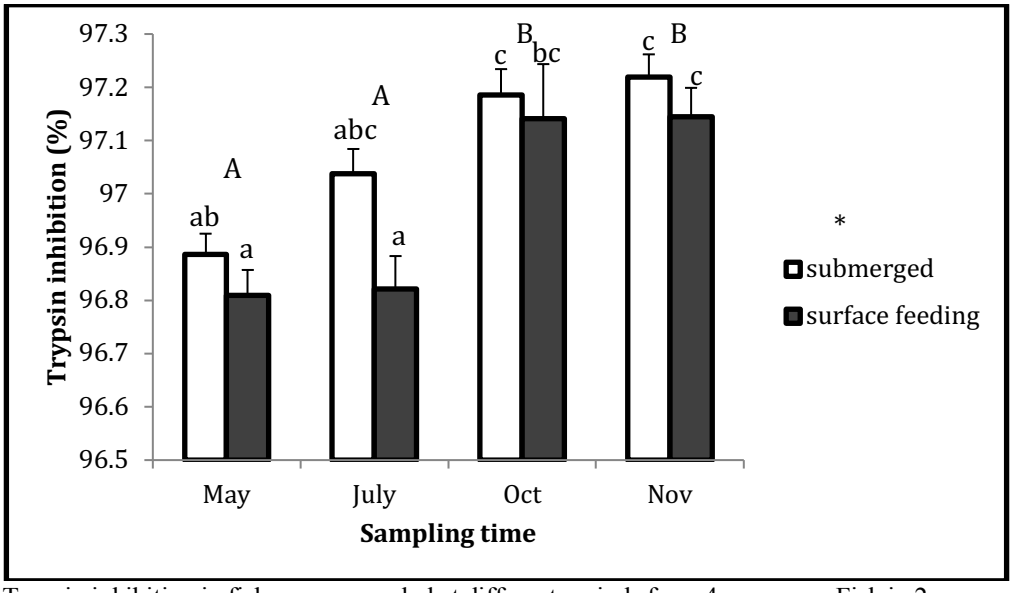

Fig. 3.4.3.3: Trypsin inhibition in fish serum sampled at different periods from 4 sea cages. Fish in 2 cages were fed from the surface (open bars) while fish from the 2 other cages were fed from beneath the surface (black bars; submerged feeding). Different uppercase letters show significant differences between sampling periods (General Linear Method, P= 6.10^{-9}). Asterisk show that there was a significant difference between the 2 feeding types (GLM, P= 0.015). Different lowercase letters show significant differences between sampling x feeding time (GLM, P=0.459, One-Way ANOVA P=4.10^{-8}, Tukey t-test). n=20.

Generally, submerged feeding provided fish with a more stable immune status than fish fed at the surface, reducing or at least delaying seasonal variations.

3.4.4 Behavioral monitoring

Regarding the behaviour of the groups, in terms of their vertical distribution in cages the pattern was similar to the one observed during previous experiments in Souda cage farm. In Appendix 2 all the echograms taken during the trial are presented. A typical example is presented in (**Figure 3.4.4.1**).

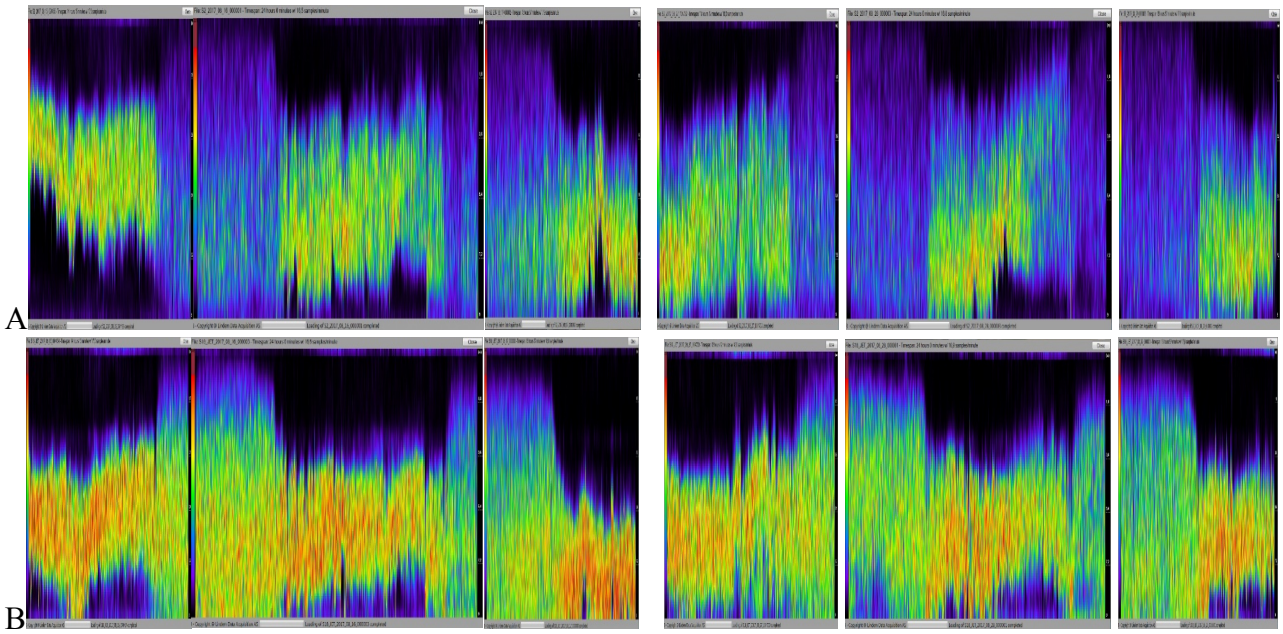

Figure 3.4.4.1. Echograms with the vertical distribution of the reared groups in (a) Standard feeding and (b) Submerged feeding. Horizontal Axis is the time while Vertical is the cage depth (from bottom to top). Different colors (from violet to red) represent different densities.

As in previous trials the observations resulted in similar pattern, 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 dark. This pattern is independent of the parameter tested.

3.5 Comparison of automatic and demand type feeding in tanks.

3.5.1 Growth and size variation

Growth between the two treatments was similar (**Fig. 3.5.1.1**). Fish in the two feeding systems, programmed and auto-demand, grow significantly (P<0.05) respectively from 58.0 ± 0.9 g and 58.5 ± 1.0 g to 340.5 ± 9.5 g and 322.1 ± 7.6 g. There were no significant differences in weigh of the groups from the two treatments during 10 months. However, on two months the programmed feeding groups were significantly (P<0.05) larger than the auto-demand groups. In March 2017, the mean weight of the fish with the programmed feeding system was 188.2 ± 3.5 g compared to 171.4 ± 3.4 g with the auto-demand feeding system and in May the programmed feeding system was 268.1 ± 5.4 g compared to 251.2 ± 5.9 g with the auto-demand feeding system. The different replicas of the same treatments were held in different recirculation systems and there was no significant interaction between the effect of the treatment and recirculation system on growth. However, the recirculation system did have a significant effect on growth and in the months February, March, May and June the fish in recirculation system 2 were significantly (P<0.05) larger than the other recirculation systems. No differences amongst recirculation systems were observed in any other month.

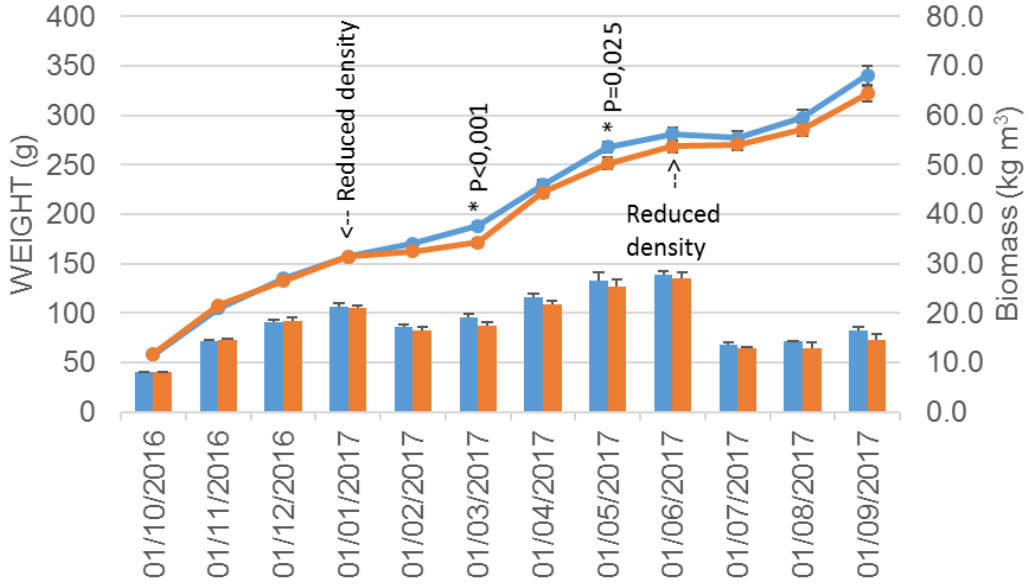

Figure 3.5.1.1 Growth in mean wet weight (lines left axis) and tank biomass (columns right axis) of meagre (*Argyrosomus regius*) fed with two feeding systems, programmed (blue) and auto-demand (brown) (n=180, 60 x 3 replica tanks). Arrows indicate when density number of fish was reduced, in January from 200 to 150 and in June from 150 to 75. An asterisk indicates significant differences between feeding treatments with the actual P value.

The growth of the fish in the two feeding systems exhibited a similar pattern over the year (**Figs 3.5.1.1** and **3.5.1.2**). There were no significant differences in specific growth rate (SGR) between fish fed with the two feeding systems or between fish held in different recirculation systems. The highest growth and SGR was observed at the beginning of the experiment. As the temperatures decreased the SGR decreased to a low during February and March. The SGR increased during the spring with rising temperature before decreasing in May, June and July due to high biomass, disturbance of reducing numbers and / or high summer temperatures. The SGR increased towards the end of the trial in August and September.

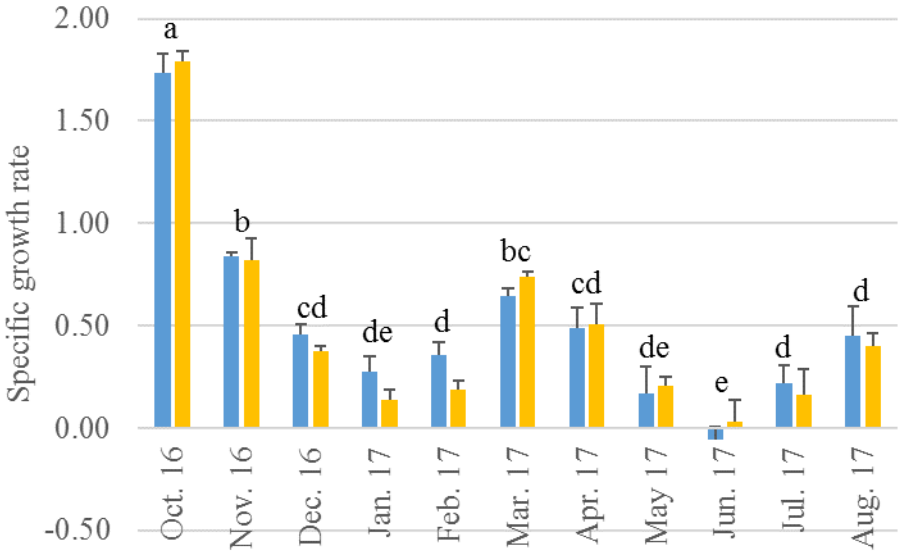

Figure 3.5.1.2 Mean specific growth rate of meagre (*Argyrosomus regius*) fed with two feeding systems, programmed (blue) and auto-demand (yellow) (n=3 replica tanks). There were no significant differences between feeding system (P=0.51). Different letters for each month indicate significant differences (P<0.05) between months for the global SGR (combining the SGR of the different treatments n=6).

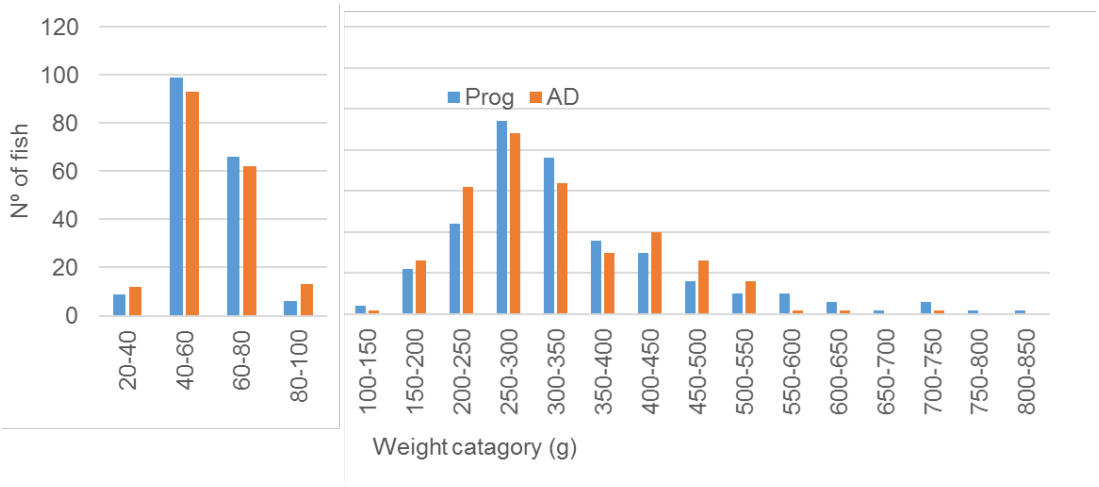

Figure 3.5.1.3. Weight distribution at the start of the experiment, 4 Oct. 2016 (left) and the end, 6 Sept. 2017 (right) of meagre (*Argyrosomus regius*) fed with two feeding systems, programmed (blue) and auto-demand (brown) (n=180 fish, 60 from each replica tank, n=3). There were no significant differences between the distribution from each feeding system on each date.

The size variation in the tank changed over the experiment. Initially in October the size distribution was normal. As the experiment progressed, the size distribution became positively skewed towards a few large fish (**Fig. 3.5.1.3)**. The degree of skew increased as the experiment progressed and when the experiment finished the distribution was highly positively skewed. The size distributions and coefficient of variation were similar and there were no significant differences in each month in the size distribution or mean CV (**Figs. 3.5.1.3** and **3.5.1.4**) between the fish populations fed with the two feeding systems, programmed and auto-demand.

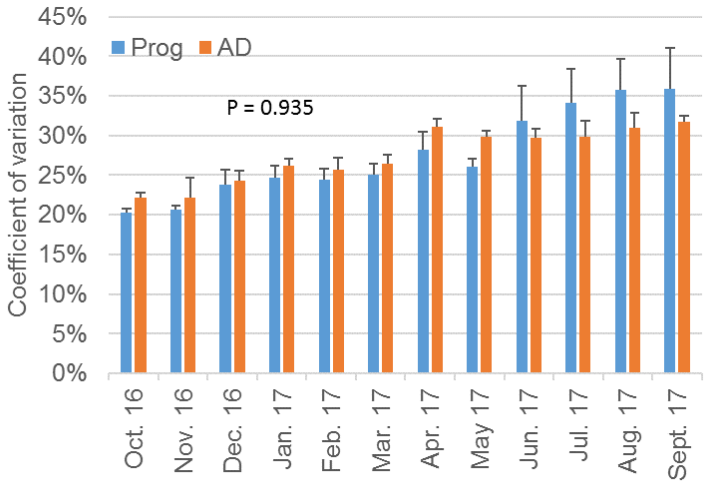

Figure 3.5.1.4. Mean coefficient of variation (CV) of meagre (*Argyrosomus regius*) fed with two feeding systems, programmed (blue) and auto-demand (brown) (n=3 replica tanks). There were no significant differences between feeding system (P=0.935).

3.5.2. Feed conversion ratio and feeding

Generally all the feed delivered to the tanks by both feeding systems was eaten and uneaten feed was not observed in the tanks or recovered from the central outlet. However, there were periods and incidents when feed delivered to the tanks was not eaten and was syphoned from the tank bottom and/or feed was observed collected in the outlet tube. During January and particularly February, feed was often syphoned from the tanks in the programmed feeding treatment. During June, after the stocking density was reduced from 150 to 75 fish per tank, all tanks had days when feed was on the bottom and growth was low or fish lost weight. This problem persisted into July for three tanks, both tanks in recirculation system 3 (Prog 3 and AD 3) and the auto-demand tank in recirculation system 2 (AD 2). Lastly, on some occasions the auto-demand feeders released the entire contents of the feeder during the night. This problem was encountered for tank AD 3 in January and July and AD 2 in July. Therefore, the FCR for the tanks on these months did not represent a feed conversion of eaten feed and included large amounts of uneaten food. For all tanks during June and the three tanks (AD 2, AD 3 and Prog 3) in July the FCR has not been included as values were negative or very high (>5) and lost meaning as an FCR.

There were no differences in FCR between fish feed with the two feeding systems, programmed and auto-demand. There was no difference over the entire experiment and the fish with programmed feeding had a mean FCR of 1.50 ± 0.02 and fish with auto-demand feeding had 1.42 ± 0.01. There were also no differences within each month and fish feed with the two feeding systems exhibited similar FCRs ranging from less than 1 during October and March to a high of more than 3 in August (**Fig. 3.5.2.1)**. The FCR above 1.5 coincided with the extremes in temperature (15ºC and 25ºC) (**Fig. 2.5.1.2**) and / or the highest stocking densities >20 kg/m^3 (**Fig. 3.5.1.1**). The reductions in numbers to reduce stocking density also appeared to impact negatively on feeding and growth particularly in June.

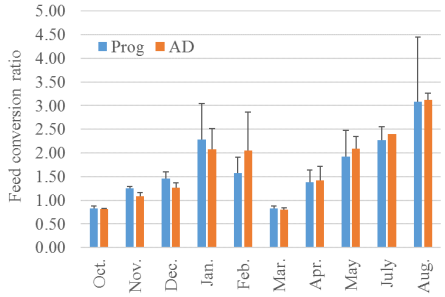

Figure 3.5.2.1. Mean feed conversion ratio (FCR) of meagre (*Argyrosomus regius*) fed with two feeding systems, programmed (blue) and auto-demand (brown) (n=3 replica tanks). There were no significant differences between feeding system (P=0.252).

The fish in the programmed feeding system were fed either three times a day or twice a day as is the practice in cage culture. The feeding time lasted one hour (as in cage systems) and consisted of delivering an equal ration of feed every 15 minutes for one hour. From 50 – 100g, which was during October, the fish received three feeding periods, 09:00-10:00, 13:00-14:00 and 17:00-18:00. From 100g and larger (November until the experiment ended) the fish received two feeding periods at 09:00-10:00 and 17:00-18:00. In comparison, the fish in the auto-demand tanks demanded feed at any time during the day and were observed to demand feed during the entire 24 hour period (**Fig. 3.5.2.2**). During February, when programmed fish did not eat the entire ration red night illumination and video cameras were briefly installed to observe the fish feeding during the night. All demanded feed was observed to be eaten. Most pellets were eaten in the water column and any pellets that fell to the tank bottom were eaten from the tank bottom. No pellets were observed to leave the tank by the outlet and no pellets were observed in the morning in the outlet pipe. In comparison, during February feed in the programmed tanks was observed in the bottom of the tank and the outlet.

The cumulative number of demands over the entire experiment was significantly higher during the night compared to the day (**Fig. 3.5.2.3**). During the night (19:00 to 06:00) there was a mean of 720 ± 22 demands / hour compared to during the day (07:00 to 18:00) with a mean of 439 ± 22 demands / hour. The pattern of feeding during the night appeared to change from the first part of the experiment to the second part of the experiment. During the period Oct. 2016 to April 2017, more feed was demand in the first part of the night from 19:00 to 22:00 (**Fig. 3.5.2.3 - middle**), whilst during the period from April 2017 to Sept. 2017 the higher demand was the entire night period 20:00 to 08:00 (**Fig. 3.5.2.3 - bottom**).

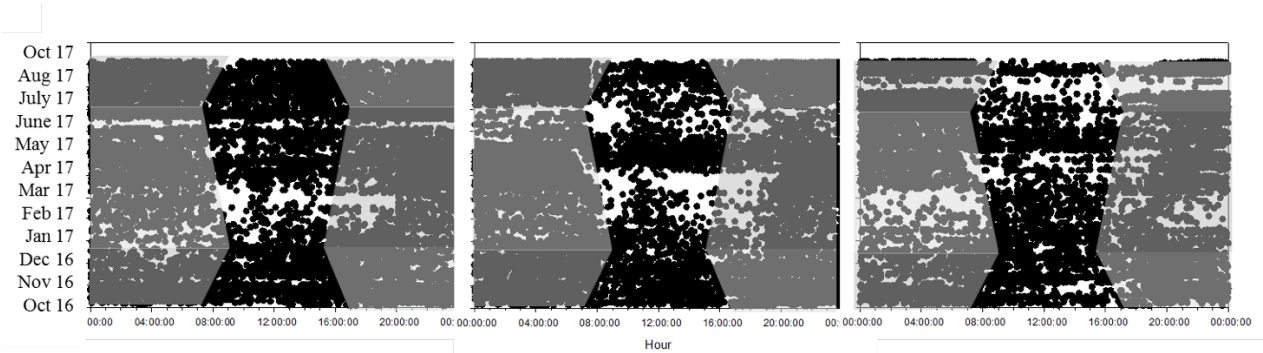

Figure 3.5.2.2 Time (x axis: 00:00 to 24:00) of feed demands (black dots) during each day (y axis: 4 Oct. 2016 to 6 Sept. 2017) of the experiment of meagre (*Argyrosomus regius*). Shaded area indicates the night period (≈0 luz) and the middle unshaded area the day period (120 ± 12 lux). The figures represent the three replicas of the auto-demand treatment, recirculation system (RAS) 1 (left), RAS 2 (middle) and RAS 3 (right).

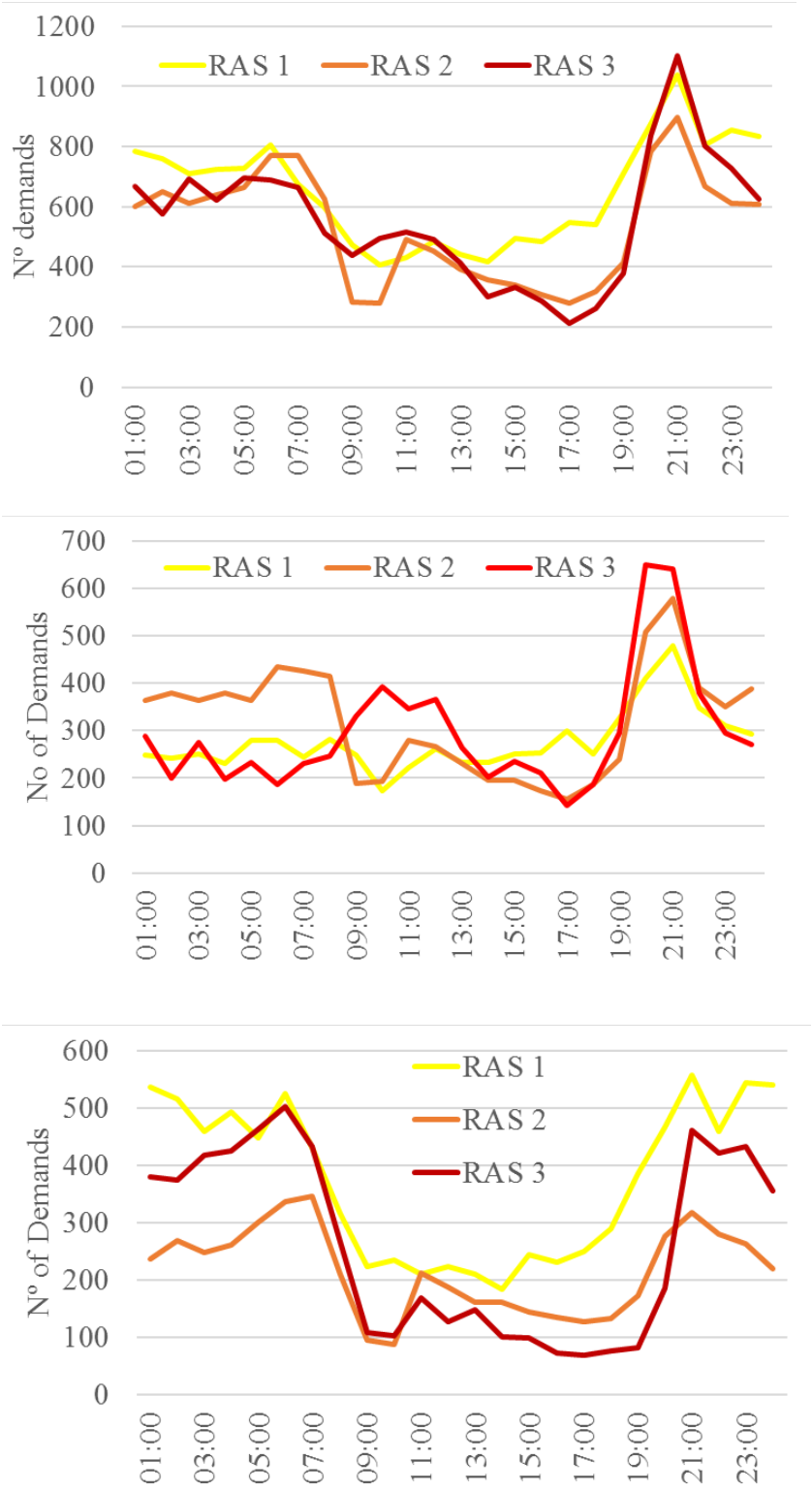

Figure 3.5.2.3 Cumulative number of demands during each hour of meagre (*Argyrosomus regius*) in three replicas in different recirculation systems, RAS 1 (yellow), RAS 2 (orange) and RAS 3 (red). The three figures represent demands during the entire experiment (Oct. 2016 to Sept. 2017) (top), during the period Oct. 2016 to April 2017 (middle) and the period April 2017 to Sept. 2017 (bottom).

3.5.3. Behaviour and fin condition

The meagre did not exhibit aggressive behaviour. During the entire experiment, punctual observations and revision of the fish during sampling indicated that meagre during the grow-out phase did not show aggression between conspecifics. The sensors at the surface (20 cm below the surface) and bottom (20 cm above the bottom) showed a clear change in behaviour during the day and night (**Fig. 3.5.3.1**). There was no difference in the distribution of counts per hour observed in the two feeding systems, programmed and auto-demand. However, in both feeding systems there was a significant difference in the distribution of counts per hour between the upper sensor (20 cm below the water surface) and the lower sensor (20 cm from the tank bottom). The fish clearly stayed lower in the tank during the day and rose to the surface to fill the whole tank during the night.

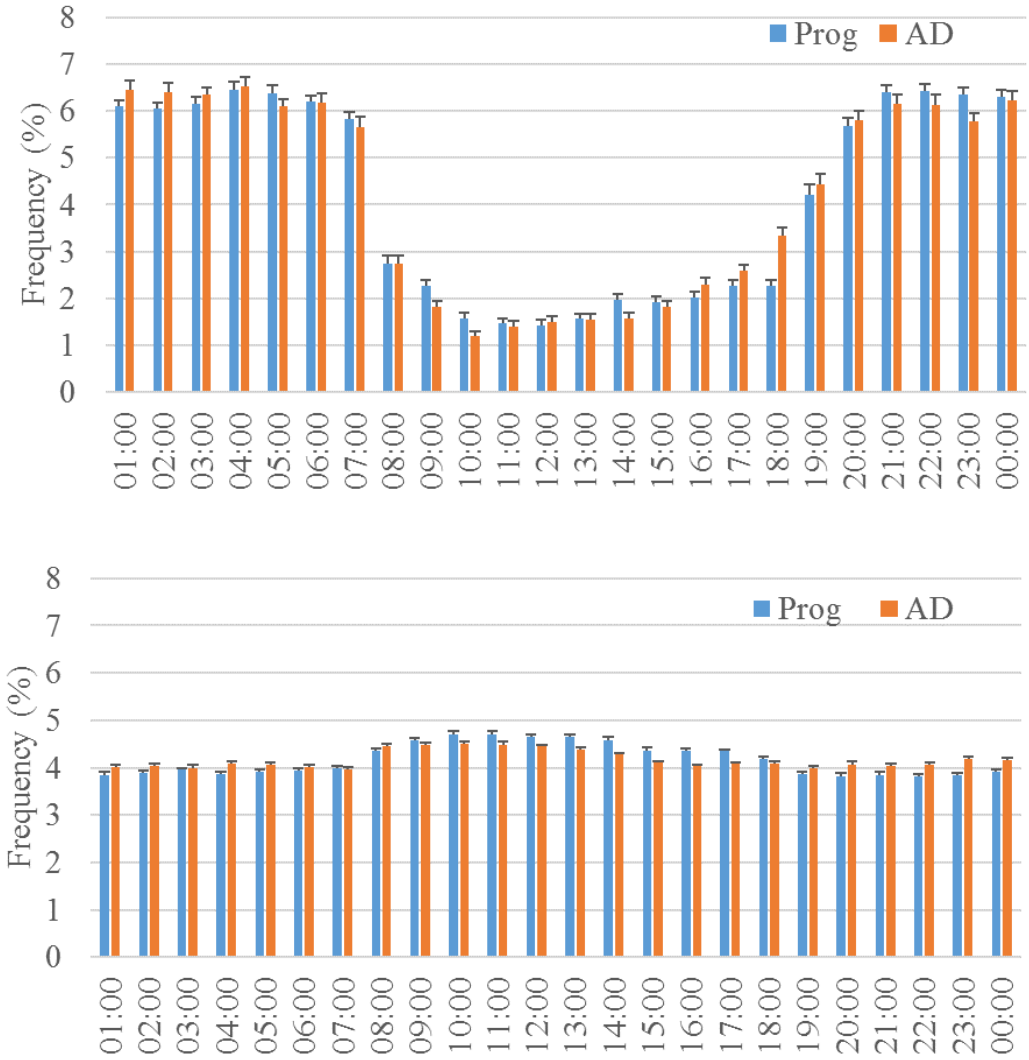

Figure 3.5.3.1 Mean percentage frequency of counts per hour for two sensors, one sensor 20 cm below the water surface (top fig.) and a second sensor 70 cm below the water surface / 20 cm from the bottom (bottom fig.) in the tanks of meagre (*Argyrosomus regius*) with two feeding systems, programmed (blue) and auto-demand (brown). Each bar represents n = 99 (3 replicas x 3 days per month x 11 months).

In the video recordings of the fish, no aggressive behaviours such chasing or biting were observed. The majority of the fish swam in a group at a similar velocity and in the same the direction around the tank. There were occasions when a fish swam in the opposite direction or with greater velocity than the group. The swimming velocity over the four days recorded, was significantly different between feeding systems. The fish in the programmed feeding system swam with a mean velocity of 37.8 ± 0.93 mm/s compared to the fish in the auto-demand feeding system that swam with a mean velocity of 31.9 ± 0.95 mm/s. The fish in the programmed feeding system exhibited a significant decline in swimming velocity during the day (**Fig. 3.5.3.2**). The swimming velocity was significantly higher (42.0 ± 2.38 mm/s) at 10:30 in the morning after the first feeding period (09:00-10:00) than at 18:30 (31.5 ± 2.05 mm/s) after the second feeding period (17:00-18:00). The swimming velocity at the other time points (08:30, 13:30 and 16:30) were intermediate with no differences amongst these or other time points. In comparison, the fish in the auto-demand feeding system had similar swimming velocity during the entire day, varying from 27.8 ± 1.49 mm/s at 08:30 to 33.9 ± 1.96 mm/s at 16:30.

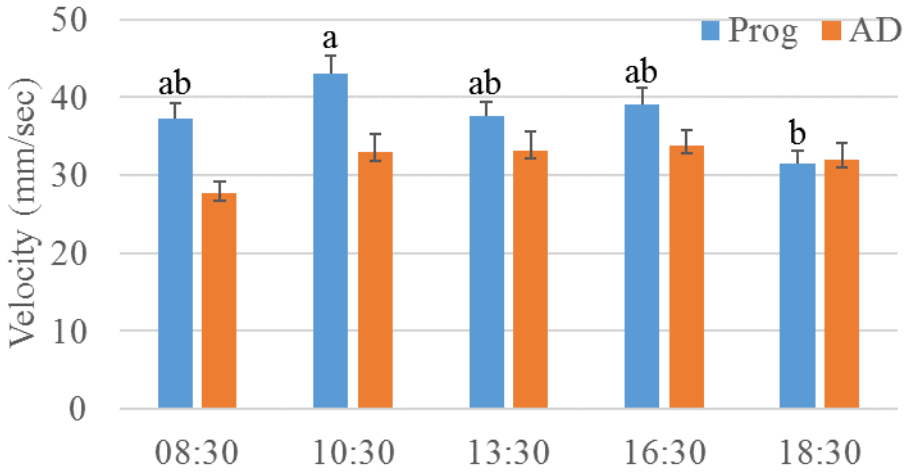

Figure 3.5.3.2 Mean swimming velocity (mm/sec) of meagre (*Argyrosomus regius*) at different times during the day in two feeding systems, programmed (blue) and auto-demand (brown). Each bar represents n = 72 (6 velocities per time point x 3 replica tanks x 4 days). Different letters above the programmed system (Prog) indicate significant differences. There were no differences amongst times for the auto-demand (AD) feeding system.

The fin condition confirmed that there was little aggression amongst the fish and generally, the fins had minor erosion that would be expected in fish in tanks with high stocking densities. There were no differences in the distribution of fin condition indices between the two feeding treatments, programmed and auto-demand. Close to all fish were classified as having index 2, light fin damage (**Fig. 3.5.3.3**), especially the dorsal (99%) and tail (100%) fins. The pectoral fins exhibited a low percentage of index 1, perfect fins (14-17%) and index 3, excessive damage (2%).

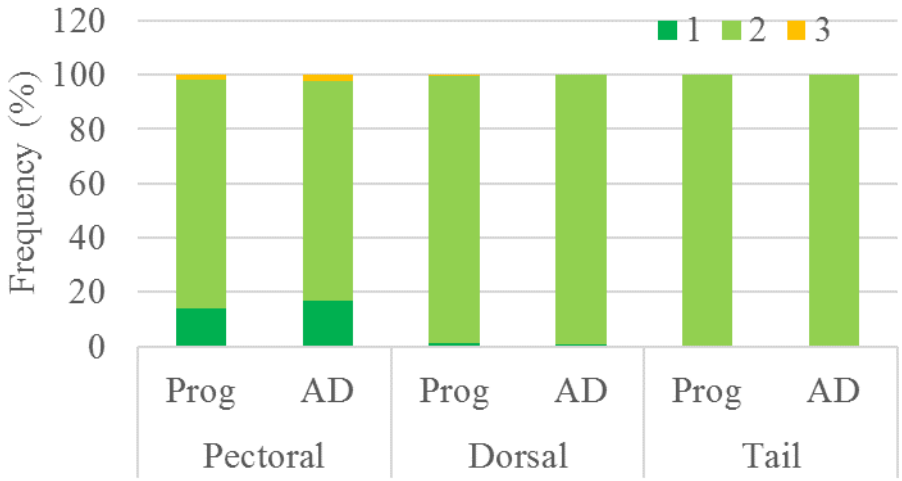

Figure 3.5.3.3 Medium percentage fin condition indices for the pectoral, dorsal and tail fins of meagre (*Argyrosomus regius*) in two feeding systems, programmed (Prog) and auto-demand (AD) during the entire experiment Oct. 2016 to Sept 2017. The fin indices were 1 = perfect fins with no damage (dark green), 2 = light damage (light green), 3 = excessive damage (orange) and 4 = no fin (no fish were observed without a fin).

4. DISCUSSION

Knowledge about the behavior of fish species of commercial interest and especially their response to different stimuli would be a fundamental tool for developing methods for improving their feeding during on growing. According to the present study, meagre feeding behavior was affected by the application of stimulus like light, air or a combination of these stimuli. Moreover, it was clear that different behavioral patterns appeared during stimuli implementation. These behavioral patterns were affected by the rearing parameters such as the size of the fish, the previous experience in different types of stimuli, the rearing conditions, such as the size of the tanks and the environmental parameters, such as light intensity.

The light stimulus can be characterized as a very strong attractant when implemented. This stimulus can be used both on naive or experienced populations, in different sizes and shapes of tanks, but also in different sizes of fish. There was a difference in response depending on the size of the fish with bigger individuals responding later than the younger to the stimuli. The limiting factor for using this stimulus is its low visibility when used under direct sunlight. Stimulus-based learning experiments for attracting fish to a particular area have taken place in other species, such as the three-spined stickleback *Gasterosteus aculeatus* (Milinski, et al., 1990) and the Atlantic salmon *Salmo salar* (Bratland, et al., 2010), where flashing light was used as a stimulus. The fading light protocol that was used with a maximum intensity of 400 lx in 20 cm distance provoked a positive reaction from the meagre rearing population as it was attracted to the feeding area during its implementation and could be further optimized as different studies showed a protocol dependent behavioral patterns of different species when light stimulus is used (Marchesan, et al., 2005). In some cases intensity of light is critical (Marchesan, et al., 2005), its polarization level, the duration of the light stimulus (Woodhead, 1966) or its spectral composition (Marchesan, et al., 2005; Reynolds, et al., 1978).

The effects of the air bubbles stimulus showed that reactions of the fish groups depended on different parameters such as the size of the fish and the previous experience in similar stimuli. The air bubble stimulus in naive populations in small meagre in 500l tanks had an adverse effect and did not attract the experimental groups to the feeding area. The fish moved away from the feeding area since they appeared to be scared during the air bubbling. In contrast, when the experiment was performed on similar tank volumes 500l and experienced fish groups, after a small adaptation period, individuals were attracted to the feeding area. The older in age and bigger in size naive individuals in 10 m^3 tanks reacted completely differently than the smaller fish and were attracted almost immediately to the feeding area during the air bubbling. Additionally, the attraction to the feeding area appeared faster at the same size individuals with air bubbles in comparison to light and was independent from the environmental light conditions as the fish moved to the feeding area at all times during the day.

In studies using air bubbles, the response of fish was shown to vary according to species. Benthic species, such as *Catostomus commersoni, Leiostomus xanthurus* and *Morone americana* were attracted by air curtains (Patrick, et al., 1985; Sager, et al., 2000), as opposed to pelagic ones, such as *Alosa pseudoharengus*, *Hypomesus olidus* and *Dorosoma cepedianum* (Patrick, et al., 1985) . These differences are probably related with the specific stimuli and experiences that each fish species receives from its habitat (Patrick, et al., 1985). The present study confirmed this observation, as in meagre, which is a benthic species, the air bubble stimulus seemed to work more effectively than the light stimulus.

In other studies using air bubbles, the degree of success in attracting or repelling the fish populations varied (Brett and MacKinnon, 2011; VanDerwalker, 1964; Warner, 1956). Also, the efficiency of

the air stimulus, when it is used as a curtain, may depend on the ability of the fish to see the bubbles as well as the pressure of air that is used (Enami, 1960). There are still indications that fish not only see the air stimulus but also can hear it, since air bubbles cause low-frequency noise that the fish can relate, positively or negatively, to various physical events such as rainfall or waterfall (Kuznetsov, 1971).

For the bigger individuals in the outdoor tanks the air bubble stimulus was more efficient, since it affected faster the learning procedure and the meagre response, than the light. The greater efficacy of the air bubble stimulus becomes even more evident from the results of feeding during noon when the individuals were mainly concentrated in the feeding area. Air bubbles essentially are a visual stimulus but at short distances they can act as mechanical stimuli as well, through the lateral line mechanic receptors of the fish or as an important acoustic stimulus for the fish, which can be perceived from long distances (Popper and Carlson, 1998). A possible explanation for the lower response to light at noon, may be the difficulty of the fish to identify the artificial light due to its low intensity (350-400 lux) compared to the natural sun light (> 24,000 lux). Therefore, the light stimulus is not considered to be suitable for use in bright lighting conditions.

The combined use of stimuli (air and light), in this study had a positive effect, attracting the fish groups in the specific area which was consistent with the results of a study using air and light as stimuli for catching fish populations (Stewart, 1981). Another study on Atlantic herring *Clupea harengus* showed that the combination of air stimulus and intense lighting attracted the fish, but it was repelled by the low light intensity (Imamura and Ogura, 1959).

In the control group it was noticed that the fish were placed relatively closer to the feeding area in most periods of the day especially during the second experiment in the small indoor tanks. This could be connected with the learning abilities of the fish but also with the previous experience with the specific stimuli of feeding. This is in agreement with other studies, where it is shown that the previous experience and knowledge of fish may have an influence on their current behaviour (Berejikian, et al., 2001; Covès, et al., 2006; Papadakis, et al., 2013; Salvanes, et al., 2007). Fish like the red porgy *Pagrus pagrus* not only had the ability of learning the place that the feeding procedure had been taking place but also the time intervals that the food needs to arrive to the feeding area, in cases that there was a delay between the time of self-feeder activation and feed arrival at the feeding area (Doxa, et al., 2011).

An additional observation that emerged from the present data was that the fish seemed to be more active when light intensity was low, during the morning and afternoon hours, whereas at noon they preferred inhabiting the shadiest areas of the tank. Meagre individuals in the present study exhibited a low diversity of cone cell types in their eyes, having mainly double cones of equal members, few double cones with unequal members and few single cones scattered on the retina surface. The low diversity in the types of cones indicates that meagre does not have great visual sensitivity and high capacity for color perception, since different size and type of cone cells is associated with different wavelengths of light absorption (Hisatomi, et al., 1996; Raymond, et al., 1993). The existence of double cones in the meagre retina indicates an increased analytical ability of the eye because the creation of double cones contributes significantly to an increase of the cone density. The arrangement of double cones in rows, as was observed in meagre, has been also observed in species that exhibit a schooling behavior, perceive their preys in a two-dimensional environment along a horizontal axis and exhibit less aggressive predatory behavior (Ahlbert, 1976; Beaudet, et al., 1997; Lyall, 1957). Indeed, meagre is a fish that tends to swim close to the bottom, and in aquaculture it frequents the

bottom of the tank or sea cage, and it tends to wait for the feed to drop to its level of swimming rather than actively swimming to the surface as soon as feeding begins.

In summary, meagre is more able to identify prey items under low light intensities and generally prefers dusky areas and low light intensity environments.

The meagre was able to learn and remember specific stimuli associated with its feeding. It responded very quickly to light stimulus (from the second day of the application). The smaller sized fish (50-100 g) in small rearing volumes require a training period in order to respond positively to the air stimulus, but in bigger fish sizes the response was shown from the second day of the stimulus application. However, under the specific conditions of the experiment, the bubble stimulus seemed to be more effective, as learning of this stimulus was much quicker, and in addition, the stimulus of light was not as effective when ambient light was strong.

With the feeding methodologies used during the present work, sufficient growth indices were presented.
The only case with insufficient growth and feed loss was the one with the automatic feeder in outdoor conditions. It seems that an important limiting factor was the light intensity that the outdoor tanks were exposed to. The high intensity during the noon resulted in altering the distribution of the reared group towards the shaded areas in the tank, leading thus to lower feeding activity and subsequent feed loss. In contrast to high light intensities at outdoor tanks, at lower light intensities in indoor tanks, the fish were more active during the whole day although at noon the feeding activity was also lower.

The main result from this trials is that fish are feeding during the whole day (all 24 h) but have an increased activity during night as shown by the results from the self-feeder. Meagre however presented no differences in growth performance or feed utilization between the tested conditions and therefore it seems that the species does easily adapt to different conditions without problem. In case of feeding with automatic feeders it should not exceed 8 hours at 19 ^{0}C respecting the digestive evacuation rate of the species.

The meagre in the present study of comparison automatic and demand feeding, over the grow-out phase from 58 g to 350 g presented a social non-aggressive behaviour towards conspecifics and an opportunistic feeding behaviour over the entire 24-hour day. The fish clearly presented nocturnal behaviour, both feeding and using the entire tank from the bottom to the surface during the night, compared to the day, when the fish remained low in the water column. However, the behaviour was not exclusively nocturnal and the fish were active and fed during the day. The feeding system, programmed or auto-demand did not have a significant effect on growth, size variation or feed conversion ratio (FCR). The aim of the trial was to determine feeding patterns that may improve growth, FCR and reduce size variation. Other species feed with auto-demand systems have exhibited improved growth, improved FCR, less size variation and less aggressive behaviours compared to systems with specific feeding times. Atlantic salmon grown with demand feeding had improved growth rates, improved FCR, reduced variation in size and reduced aggression (Noble et al., 2007, 2008). Whilst for European seabass growth and FCR were the same or improved with no difference in size variation (Boujard et al., 1996; Azzaydi et al., 1998, 1999, 2000). These studies were conducted in both tanks and cages and Noble et al. (2008), demonstrated that the results from demand feeding trials could be used to improve feed tables for fixed automated programmed feeding to obtain the same growth and FCR as obtained with demand feeding in tanks. However, meagre did not present a clearly defined feeding pattern. The meagre feed during the entire 24 hours of the day with no clear daily peak. Over the entire year the cumulative amount of feed eaten each hour did show a pattern

with elevated feeding during the night compared to the day. A mean of 720 ± 22 demands / hour were made during the night (19:00 to 06:00) compared to 439 ± 22 demands / hour during the day (07:00 to 18:00), which indicated that over the entire year 62% of the feed was demand during the night and 38% during the day. The observed feeding over the entire 24 hours of the day compared to programmed feeds at 09:00 and 17:00, did not have an effect on the growth, size variation or FCR.

There were differences in growth on two dates in March and May, when the fish with the programmed feeding system were larger than the fish with the auto-demand system. The growth advantage in March was related to superior (but non-significant) growth rates during January and February when the temperatures were at a minimum (15ºC). During this period, the fish in the programmed feeding system were overfed and feed was often retrieved from the tanks. The fish in the auto-demand demanded very little feed and consequently exhibited lower growth. It appeared that the temperatures of 15ºC suppressed appetite and growth, but that overfeeding improved growth compared to natural low feeding. The FCR in February reflected that feed was not consumed in the programmed system with a higher FCR, although there were no differences in FCR. Once the programmed fish had a size advantage, the advantage was more or less maintained for the remainder of the experiment although the variation in growth rates between the treatments generally (apart from May) resulted in no significant differences.

There were no differences in growth rate (SGR), size distribution or FCR within any month during the experiment. However, there was considerable variation in both SGR and FCR between months, indicating that factors other than the feeding system influenced the SGR and FCR in both feeding systems. The most obvious factor was the temperature and the winter low of 15ºC clearly supressed appetite and growth (discussed above). Optimal growing conditions were in October and March, with an SGR above 1.5 g/day in October and 0.75 g/day in March and FCR below 1 in both months. October had temperatures of 23ºC and stocking densities of less than 10 kg/m^3 and March had temperatures of 16ºC and stocking densities of 20 kg/m^3. However, it would appear that during the summer months growth and FCR were negatively influenced. Amongst possible negative factors were the high temperatures (25ºC from July to Sept.), stocking densities (>25 kg/m^3 during May), size of fish in relation to the size of the tanks and the reduction of numbers (January and June). It appeared that stocking density and the reduction in number impacted negatively during May and June and fish size or temperatures impacted negatively in July and August. Although at the end of the experiment mean weights were 322 - 340 g the highly positive skewed populations contained fish as large as 700 – 800 g. This size variation is similar to the situation in cage culture and Deliverable 20.1 demonstrated that faster growing juvenile fish obtain and maintain a growth advantage. The size variation was not related to feeding system.

Generally, the behaviour of the meagre was not effected by feeding system. The meagre were non-aggressive towards conspecifics, observations of the general behaviour and in videos did not identify aggressive behaviours such as chasing or biting and there were no differences in fin condition between the fish in the two feeding systems. The pectoral, dorsal and tail fins showed light fin damage that was normal for the holding condition. There was no difference in the upper and lower sensor in the tanks with different feeding systems. All fish were observed to stay low in the tank during the day and rise to fill the whole tank during the night. The only difference observed in behaviour between the feeding systems was in the swimming velocity. The fish in the auto-demand feeding system had lower swimming velocity than fish in the programmed feeding system. This appeared to be related to feeding and availability of feed. The fish in the auto-demand feeding system had feed availability at all times and did not increase swimming speed in response to hunger and/or feed delivery. However, the fish in the programmed feeding system had higher swimming speeds in response to hunger and

periodic feed delivery. This was also highlighted, as the fish in the programmed feeding system had a changing swimming velocity in response to hunger and food delivery during the day. The swimming velocity of fish in the programmed feeding system was highest in the morning (10:30) immediately after feeding (09:00-10:00) and decreased to the lowest level at 18:30 immediately after the afternoon feed (17:00-18:00) that completed the ration for the day to meet the daily feed requirement of the fish.

5. CONCLUSIONS

The meagre were non-aggressive towards conspecifics and stayed low in the cage or tank during the day, compared to the night when the entire cage or tank volume was used from the surface to the bottom. Meagre were feeding during the whole day (all 24 h) as shown by the results from the self-feeder experiments. Meagre, however, presented no differences in growth performance or feed utilization between the tested conditions (timing of feeding – continual, meals during the day or night, position of feed delivery – surface or low in the cage) and, therefore, it seems that the species easily adapted to different conditions without problem. However, despite of this adaptation to different conditions, meagre exhibited higher activity and feeding during the night. Eye morphology indicated an organism adapted for nocturnal vision. Feeding and activity was suppressed by high light intensity. Meagre that were fed at depth had improved immune parameters compared to meagre fed during the day that rose to the surface during periods of higher light intensities. The digestive system evacuated in 8-12 hours for 200g fish at 19ºC. Optimal growth and feed conversion was observed at stocking densities below 20 kg/m^3 and temperatures from 16 – 23 ºC. Stocking densities above 25 kg/m^3, temperatures <15ºC and possibly >25ºC appeared to have negative effects on growth and FCR. Together these biological characteristics recommend that meagre are fed when light intensity is low (dusk, dawn and night) and are left to digest during the hours of high light intensity (daytime– particularly mid-day).

Air bubbles and light were quickly learnt and responded to as feeding stimuli. Both air bubbles and light or a combination of the two can be used in an industrial setting, as systems to apply these stimuli can be manufactured, implemented and managed easily with existing technologies in sea cages. However, the conditions under which the rearing is carried out should be taken into account in order to select the appropriate stimulus. Thus, the use of bubbles could easily be incorporated into the rearing of meagre, whereas the light stimulus could be used in conditions where feeding is performed under relatively low light intensities (early morning, afternoon, evening), or in cases where in general the rearing takes place under low lighting, e.g. in covered tanks or cages. Additional studies using different stimuli would be useful as the general information about the behavior of meagre is considered limited. For example, it might be advisable to study the response of meagre in other types of stimuli, such as acoustic stimuli and test their effectiveness when used as stimuli for feeding.

Therefore, the optimal feeding methodology that adjusts to the biological characteristics of meagre during grow out should aim to feed when light intensity is low (dusk, dawn and night) using stimuli to ensure a good feeding response from fish that can often not be observed and the fish should be left to digest during periods of high light intensity (daytime – particularly mid-day).

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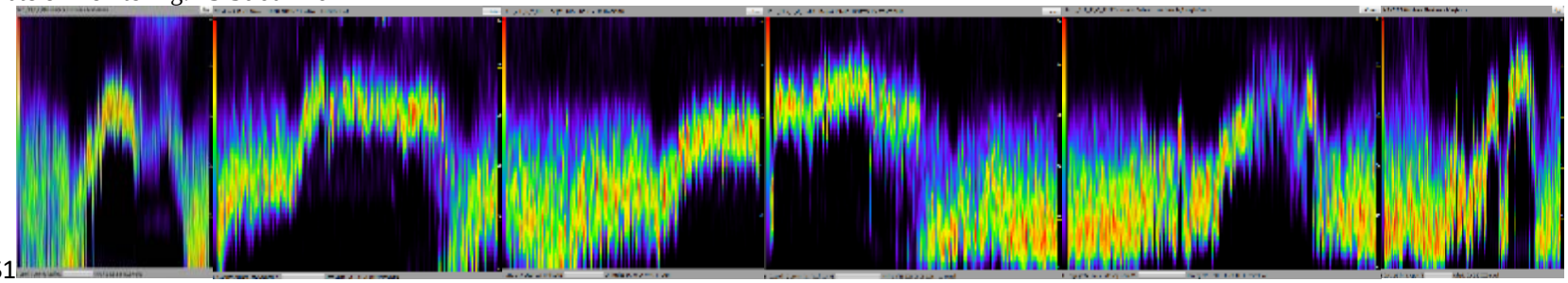
APPENDIX 1

Echographs from the experimental cages during the rial in the Souda cage farm.

Cages noted as S1 and S2 were the ones fed during day while cages noted as S17 and S18 were the ones fed during night.

Date of monitoring: 25-30 Jun 16

S1



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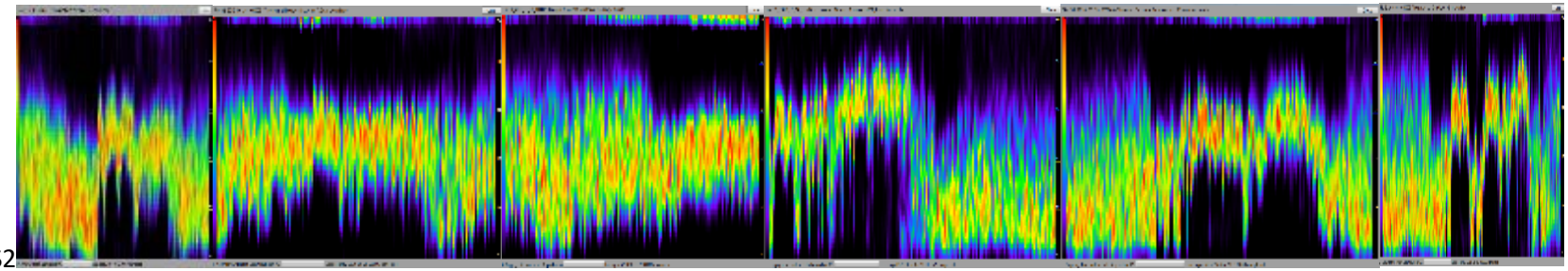

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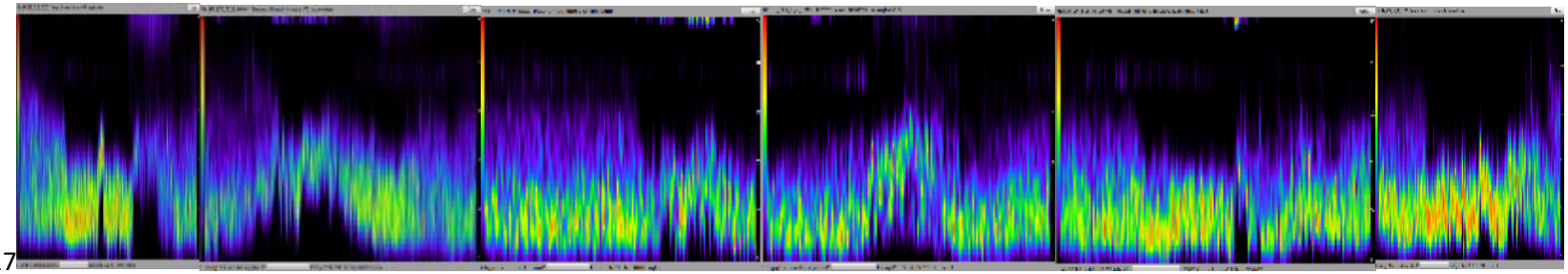
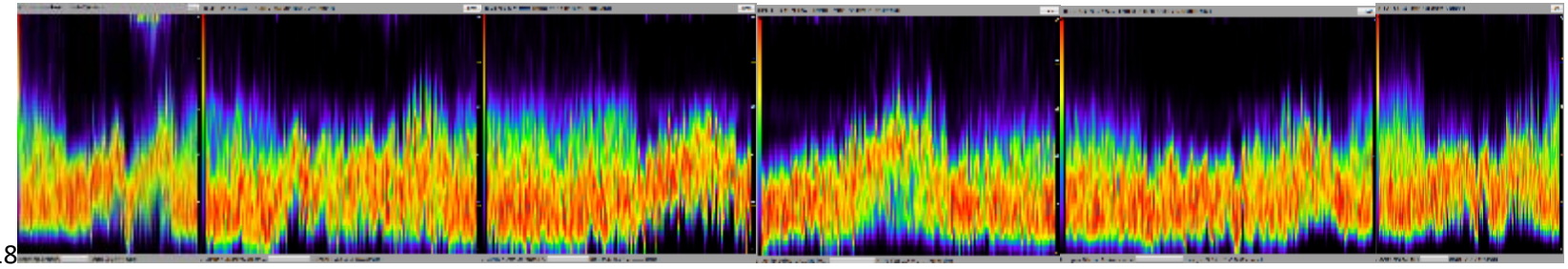
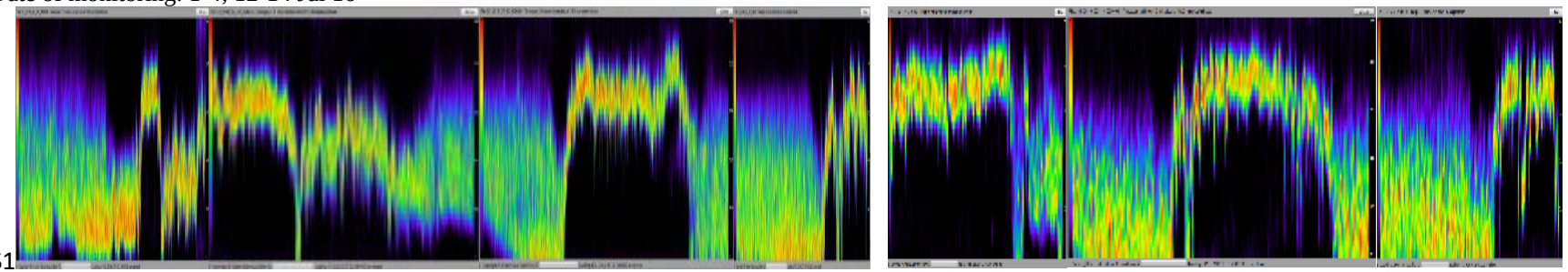
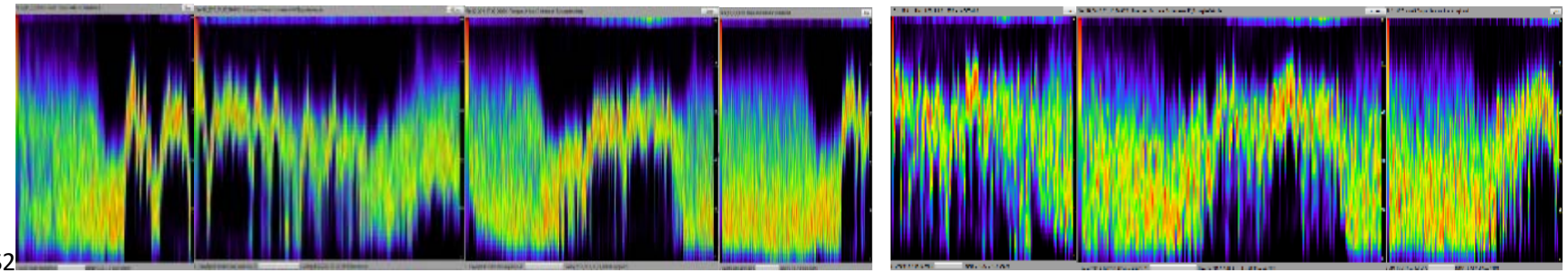


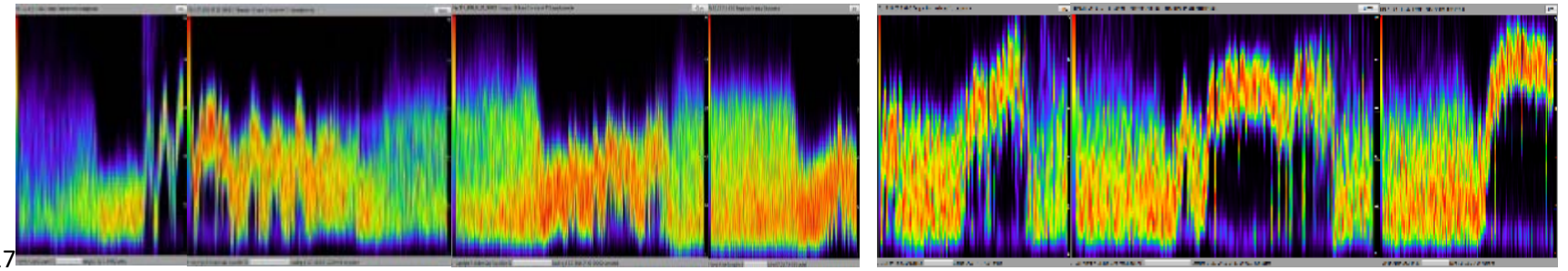
S18

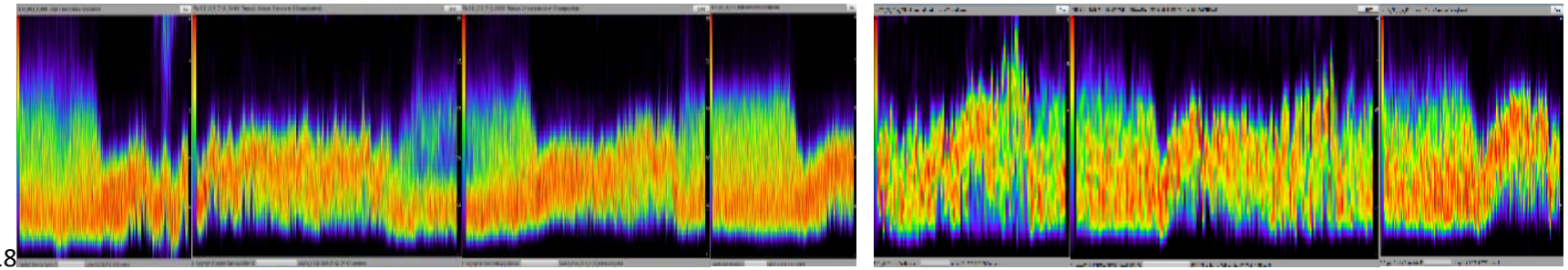


Date of monitoring: 1-4; 12-14 Jul 16

S1 

S2 

S17 

S18 

Date of monitoring: 18-21 Jul 16; 1-3 Aug 16

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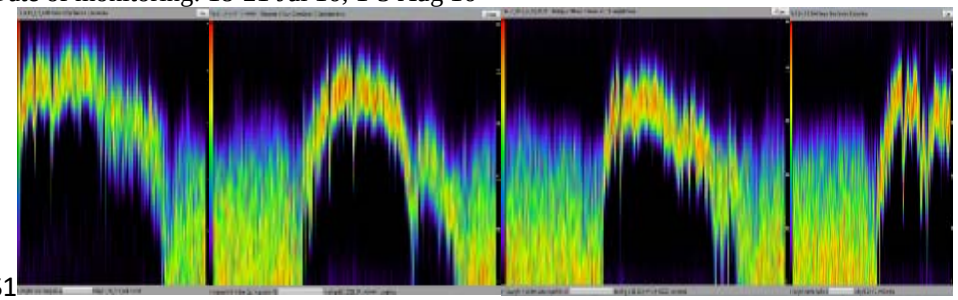

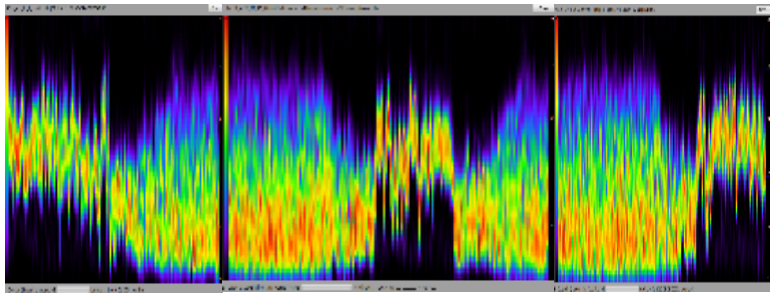

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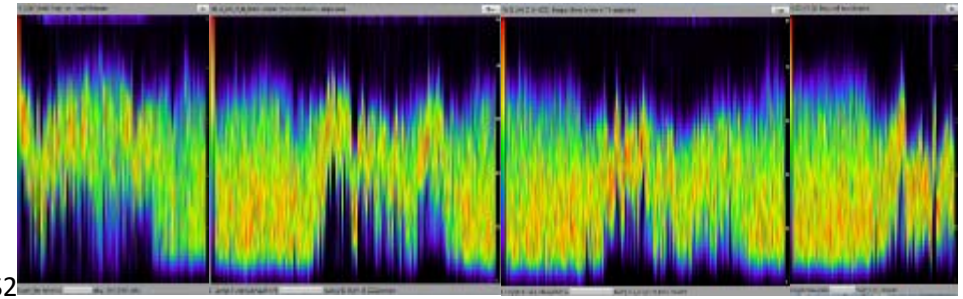

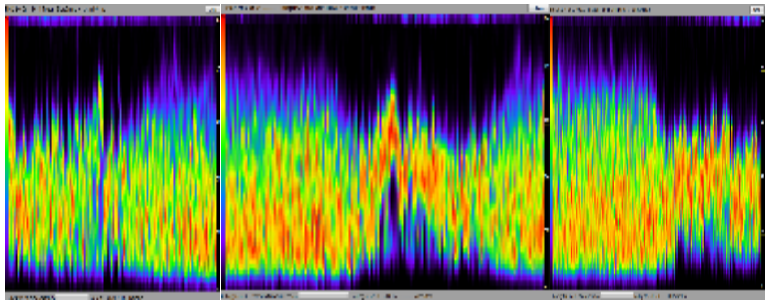

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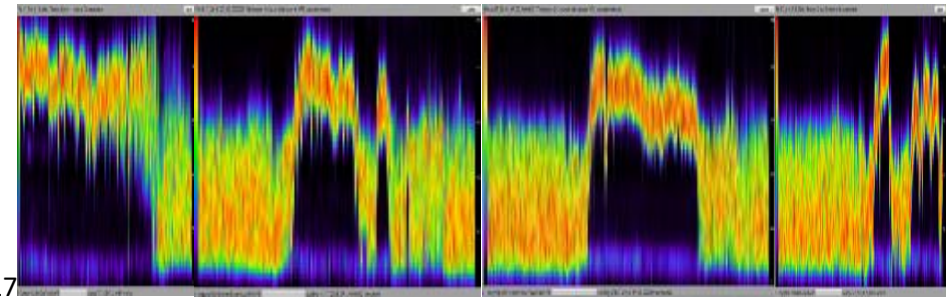

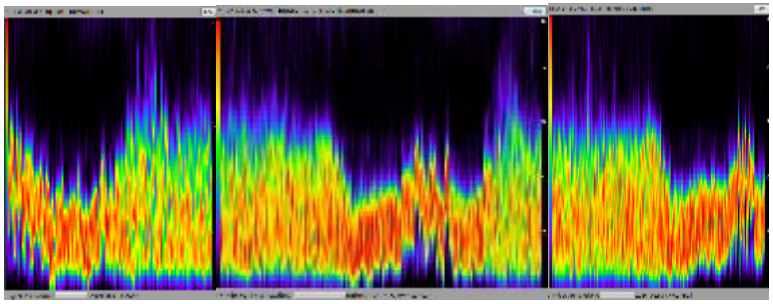

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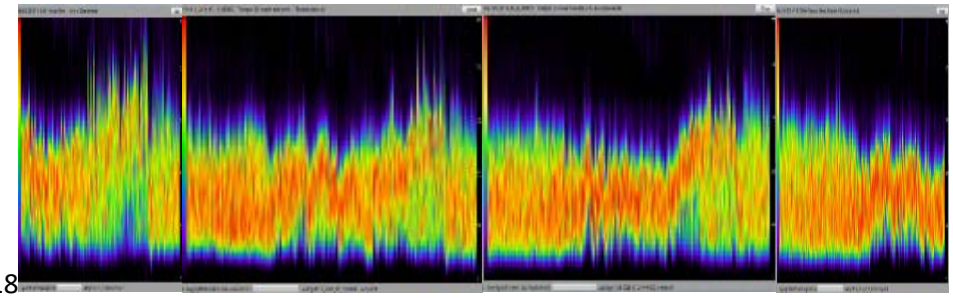
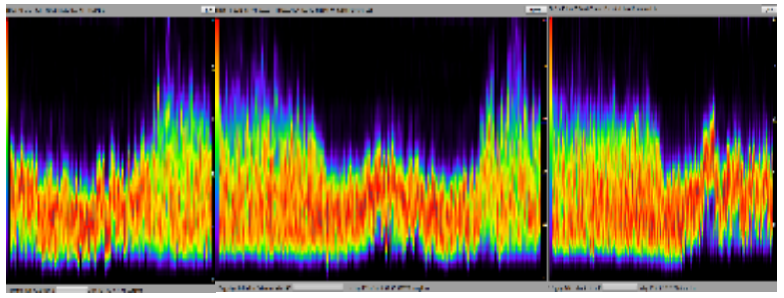
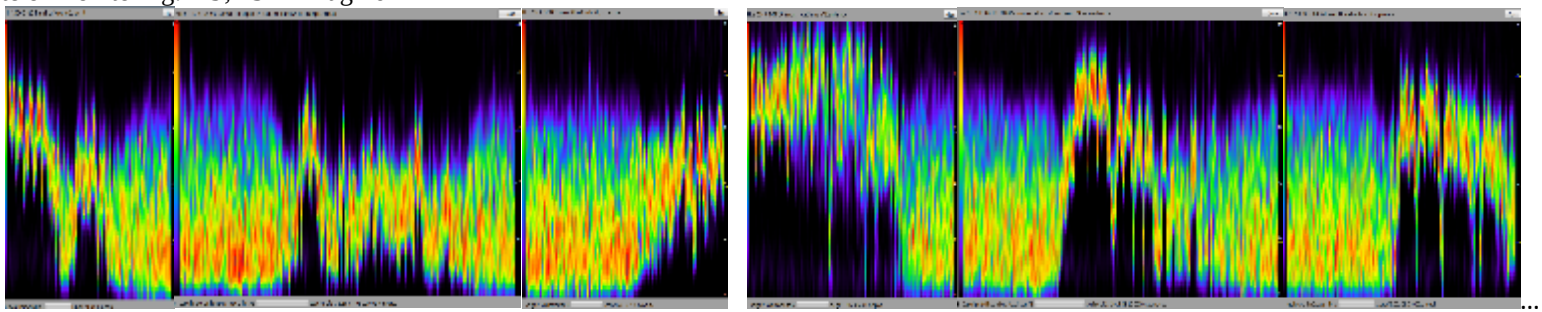
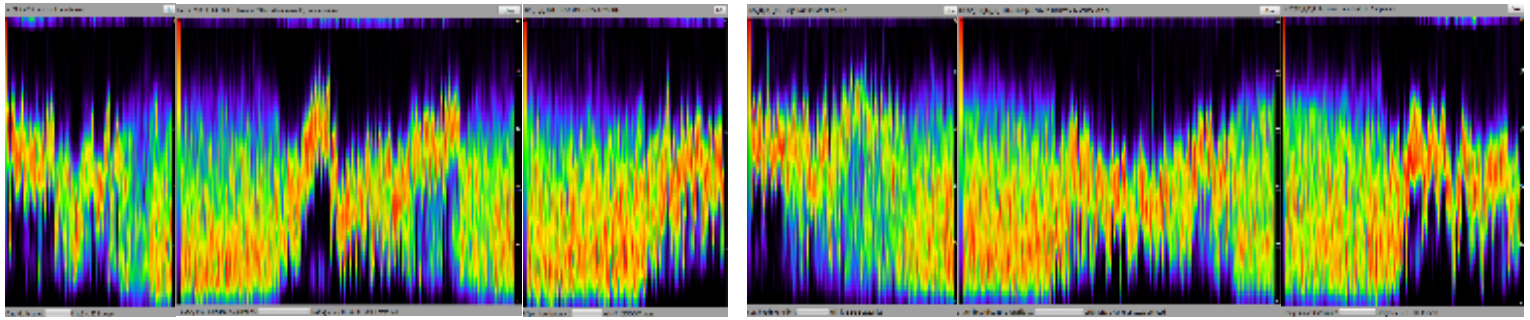
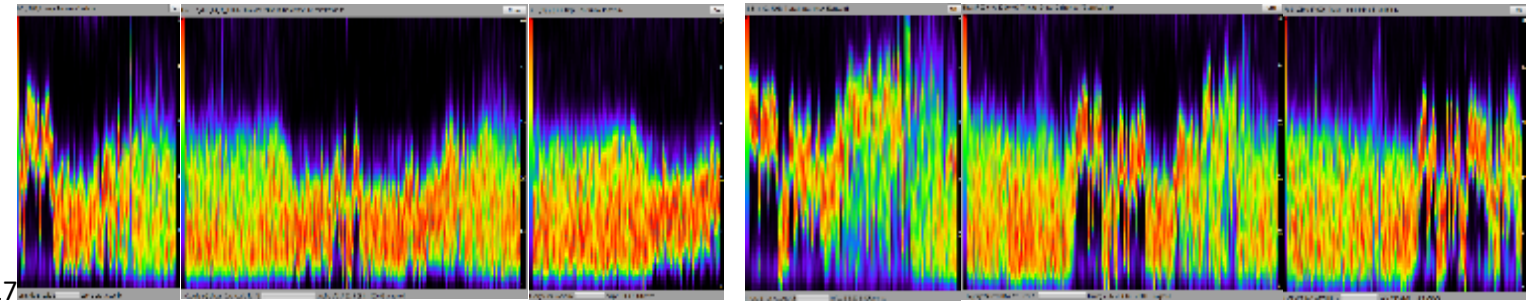


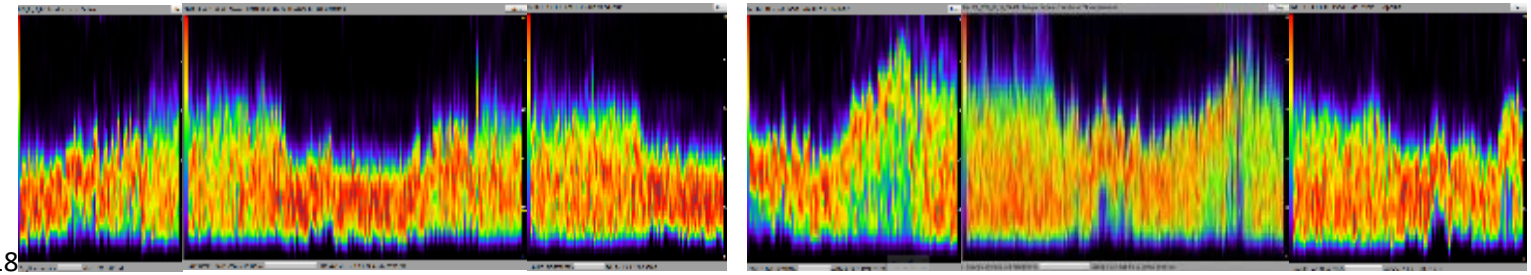


Date of monitoring: 7-9; 15-17 Aug 16

S1 

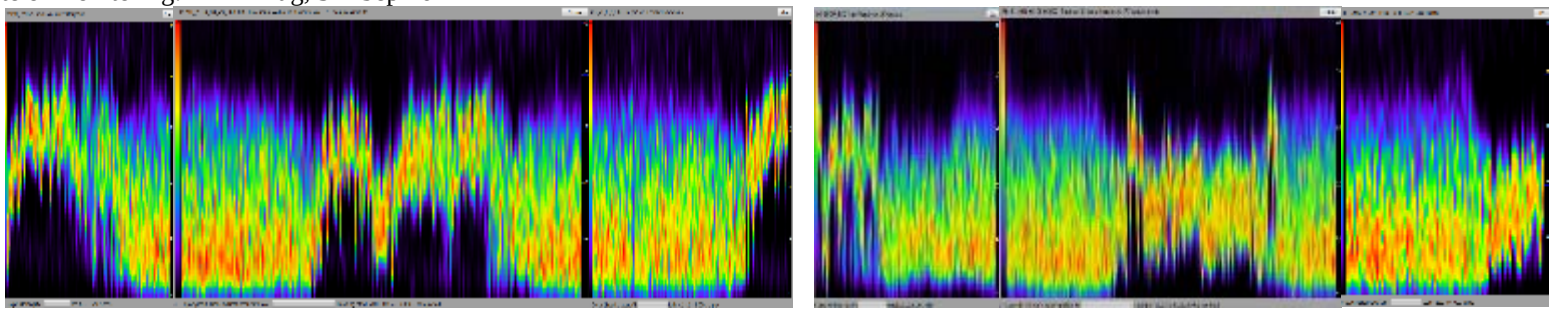
S2 

S17 

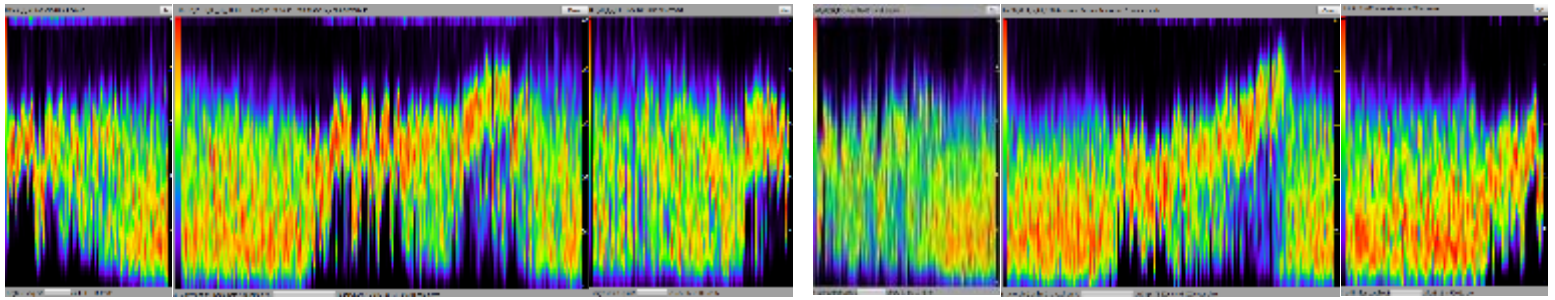
S18 

Date of monitoring: 22-24 Aug; 5-7 Sep 16

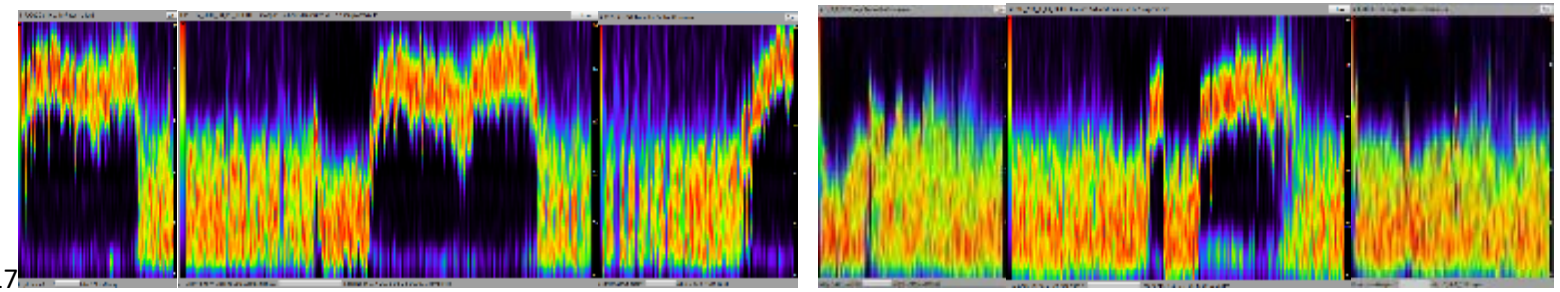
S1



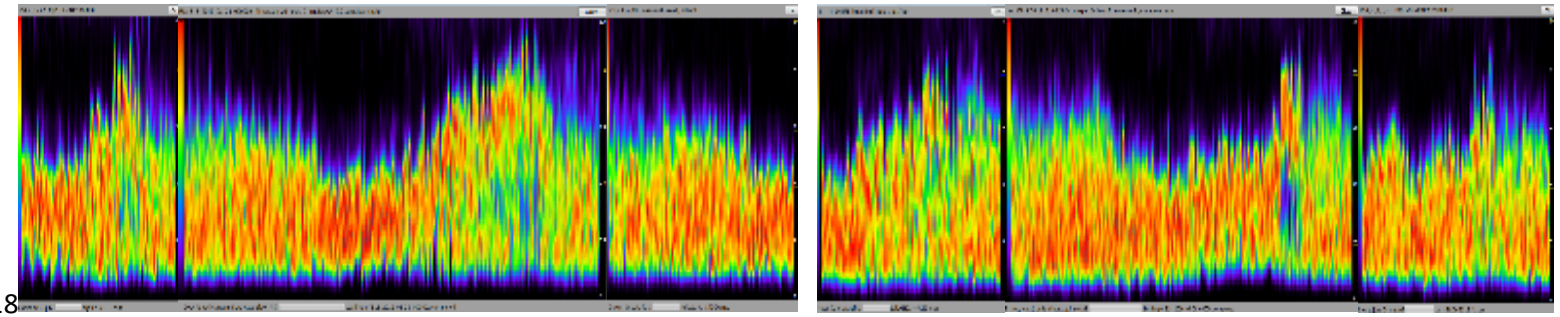
S2



S17

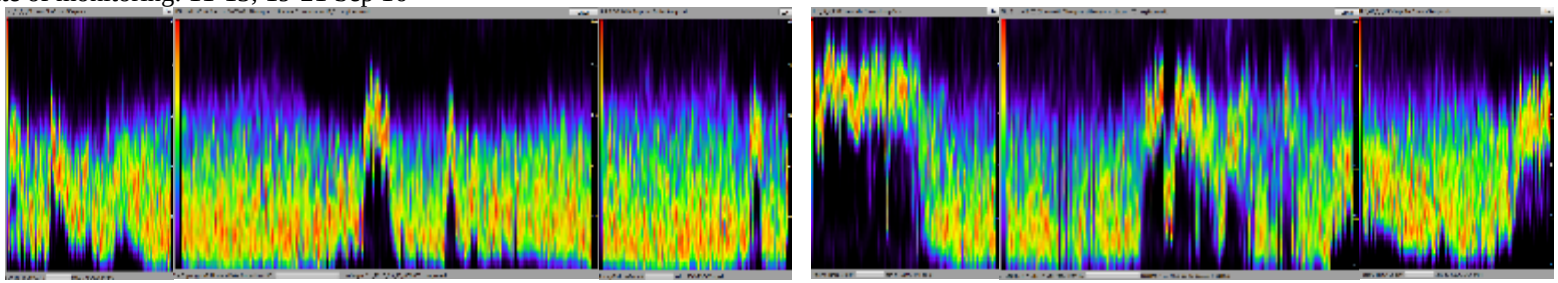


S18

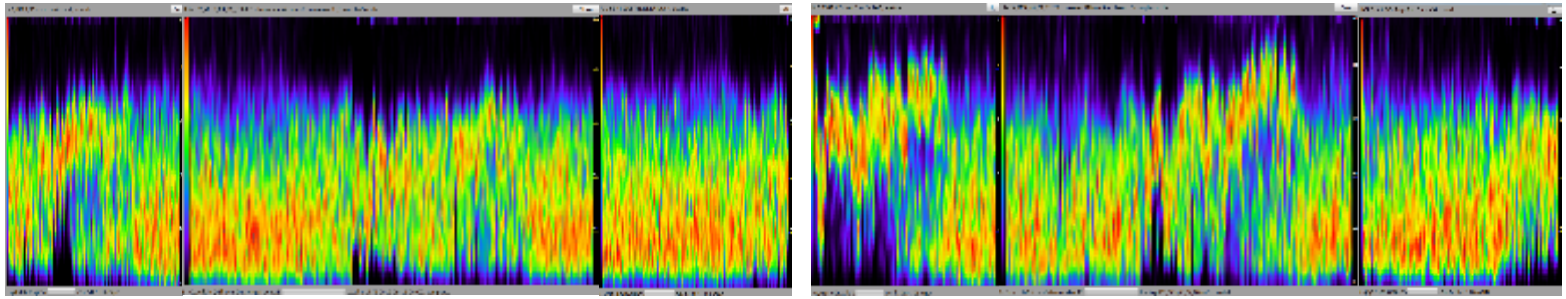


Date of monitoring: 11-13; 19-21 Sep 16

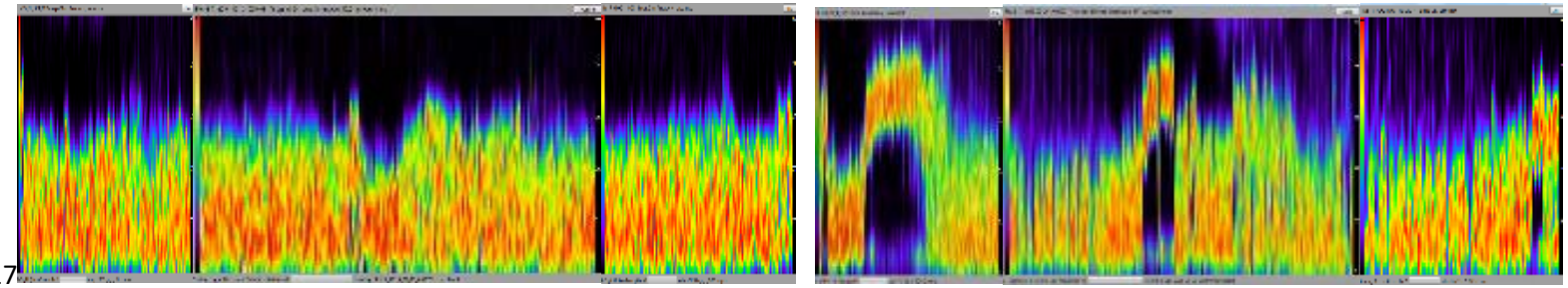
S1



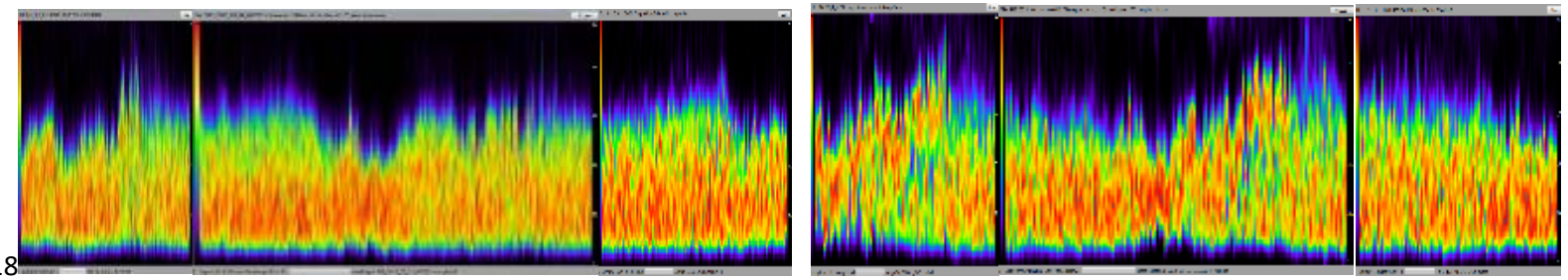
S2



S17

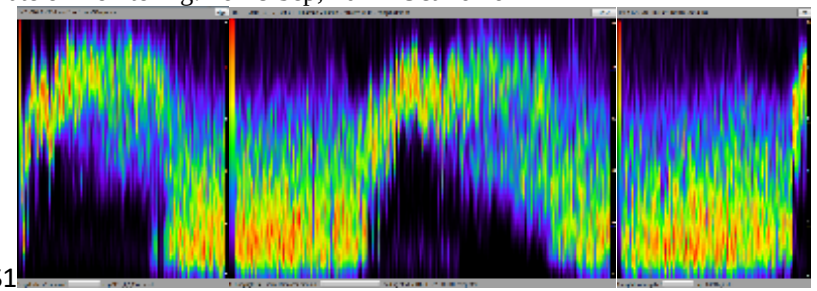


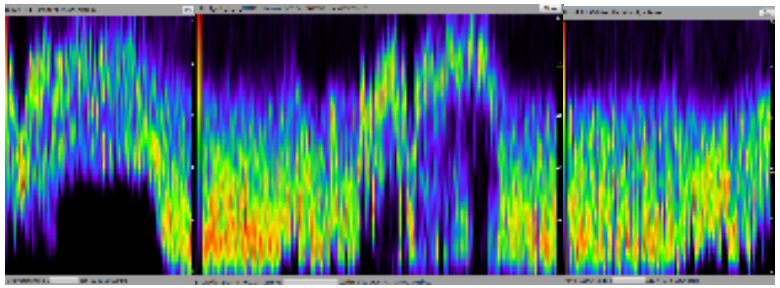
S18



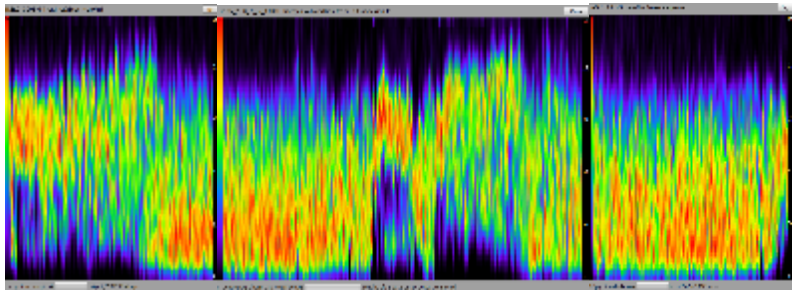
Date of monitoring: 26-28 Sep; 10-12 Oct 16 16

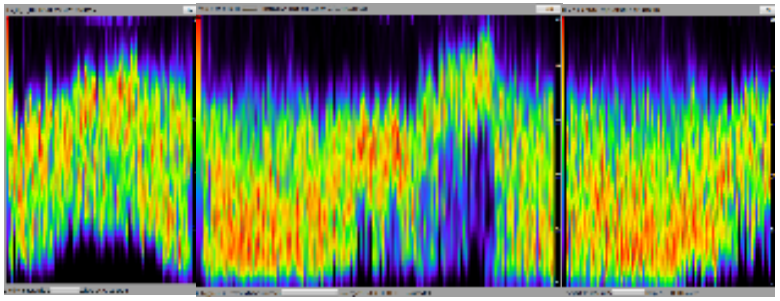
S1



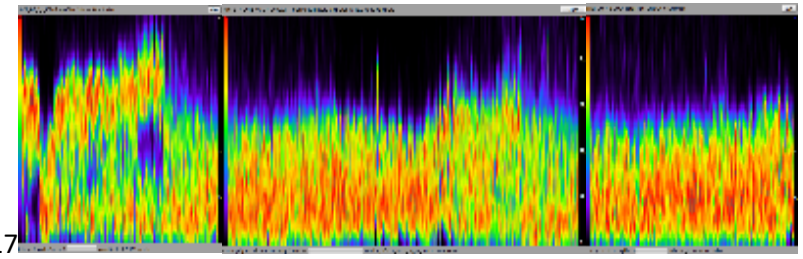


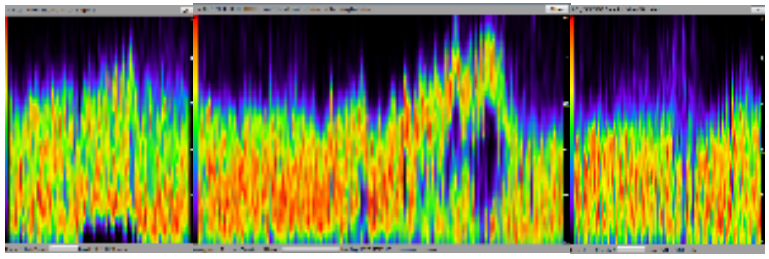
S2



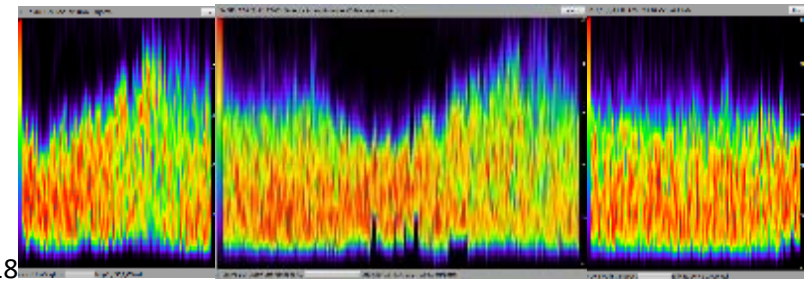


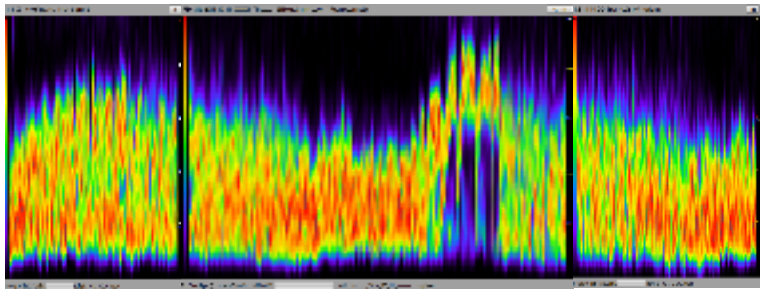
S17



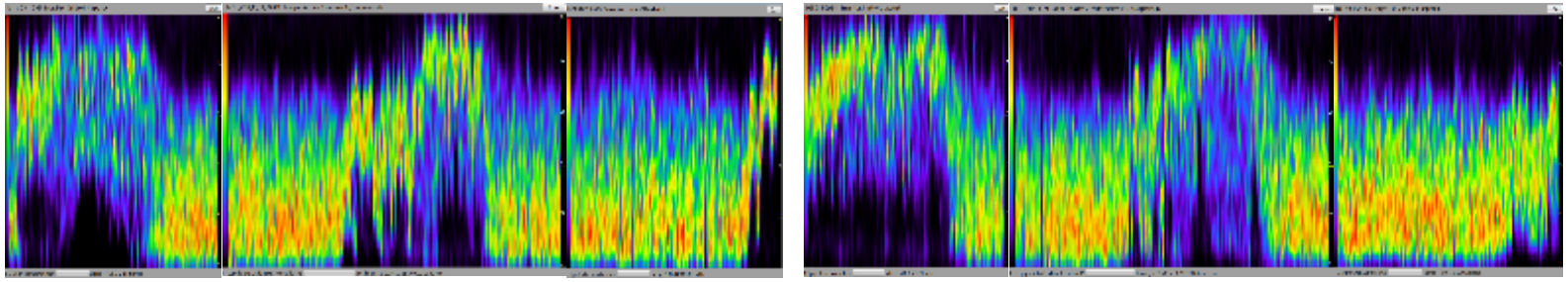


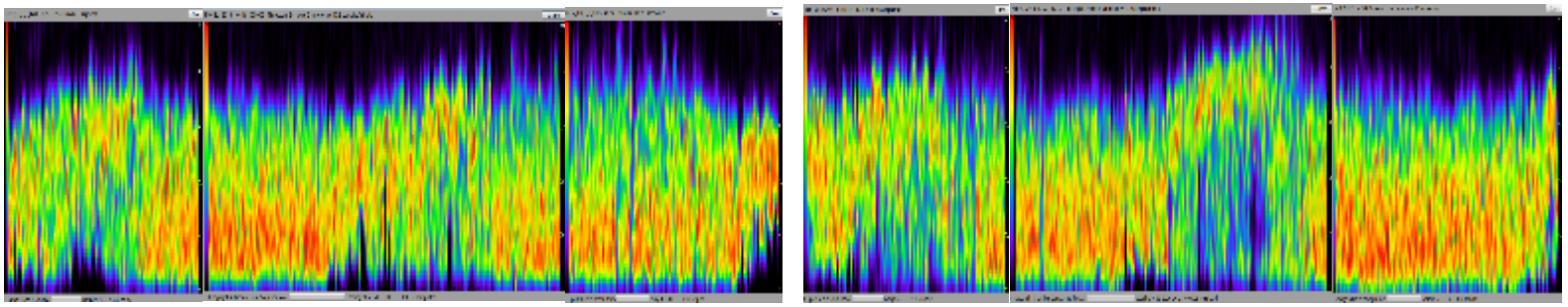
S18

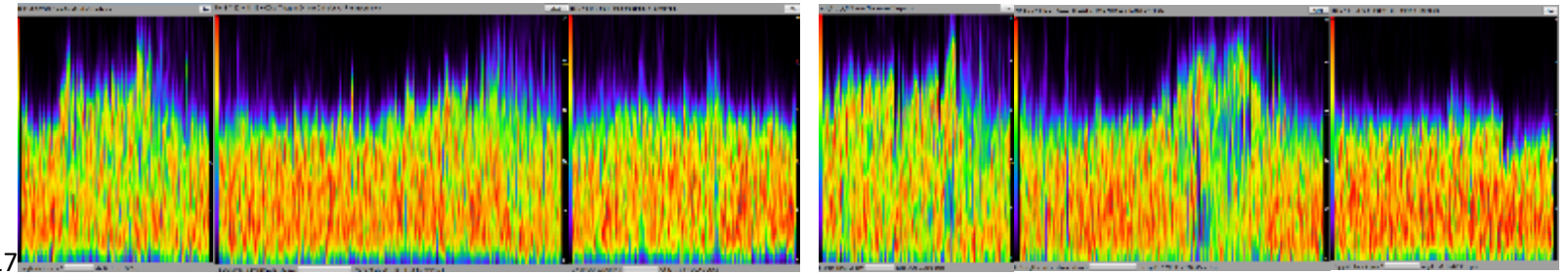


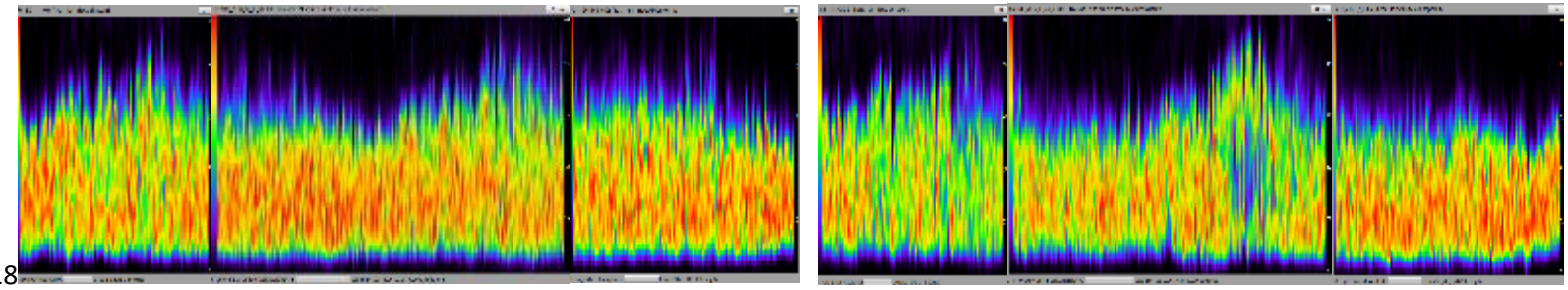


Date of monitoring: 17-19; 22-24 Oct 16

S1 

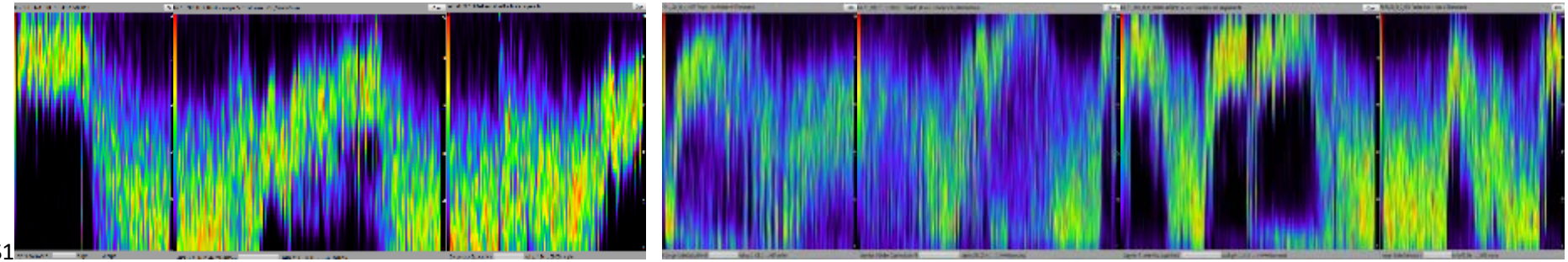
S2 

S17 

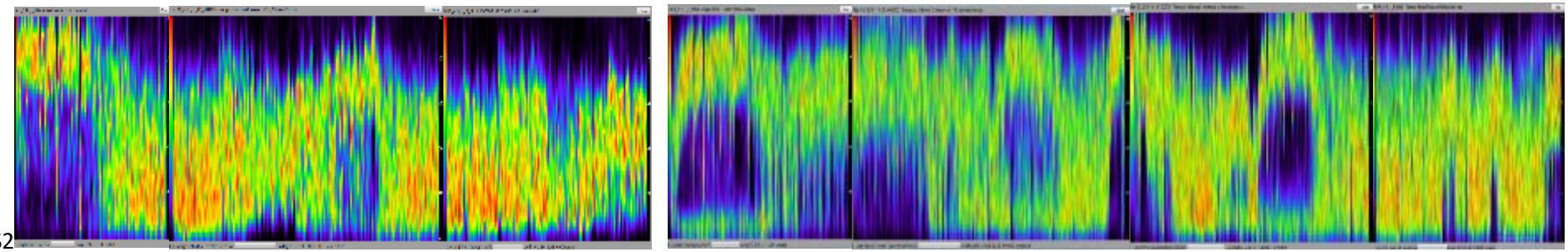
S18 

Date of monitoring: 1-3 14-17 Nov 16

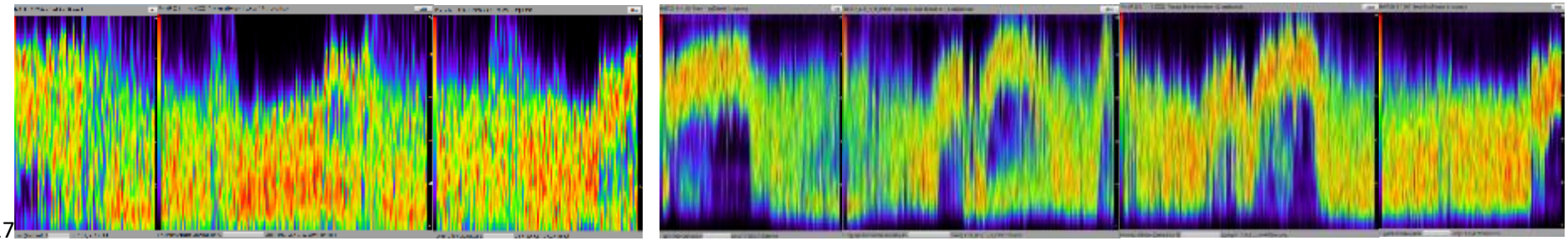
S1



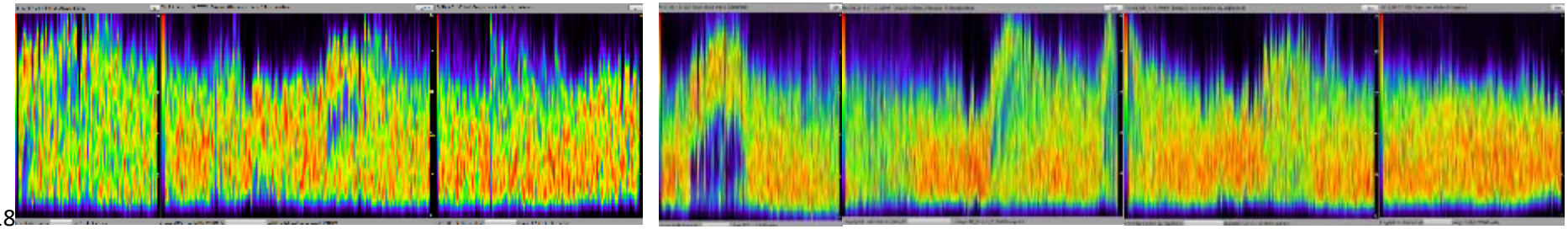
S2



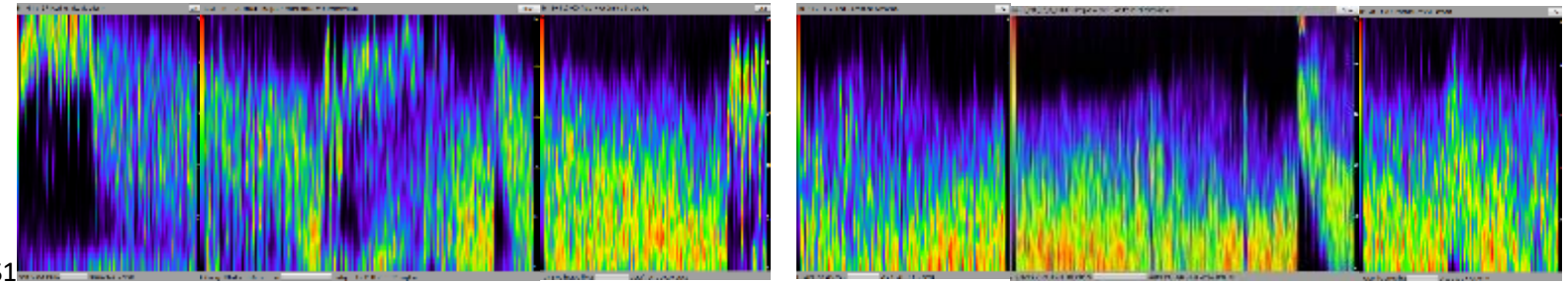
S17

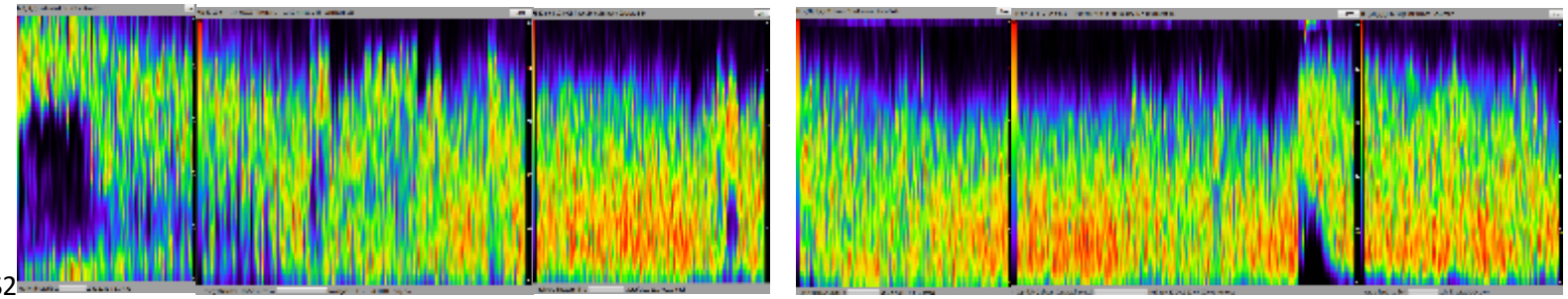


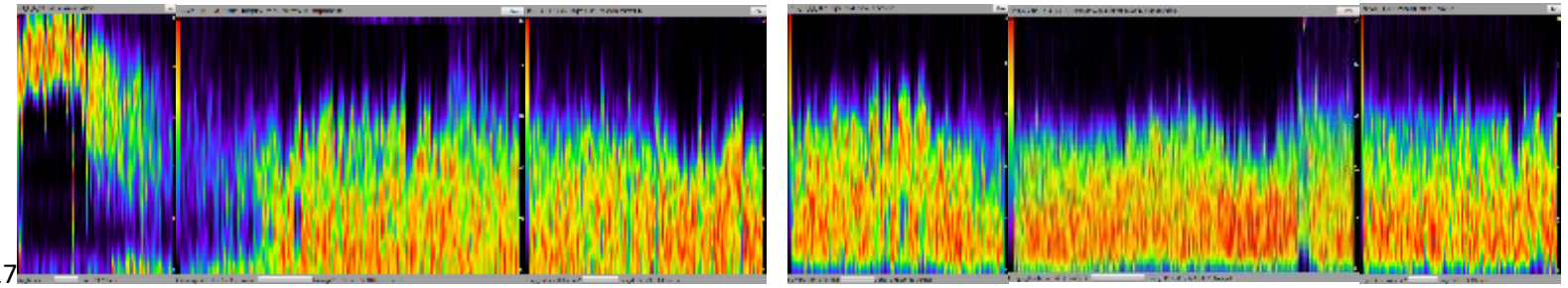
S18

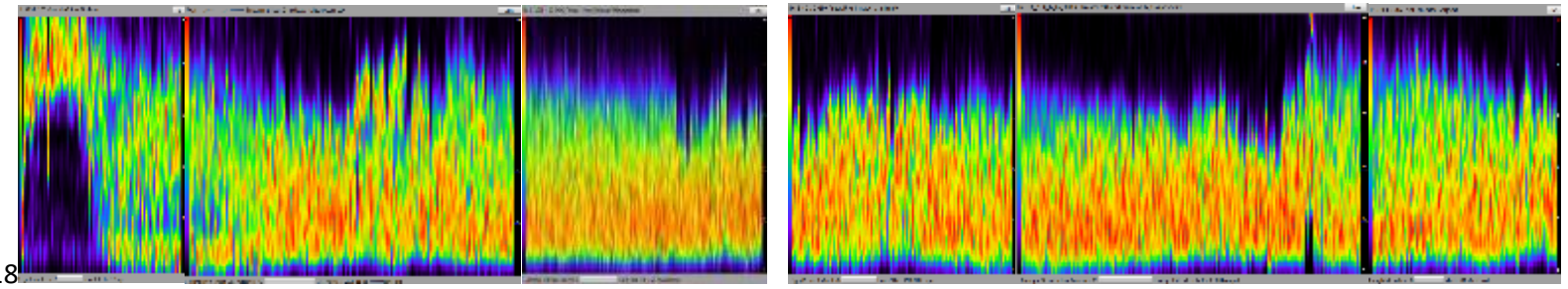


Date of monitoring: 25-27 Nov 4-6 Dec 16

S1 

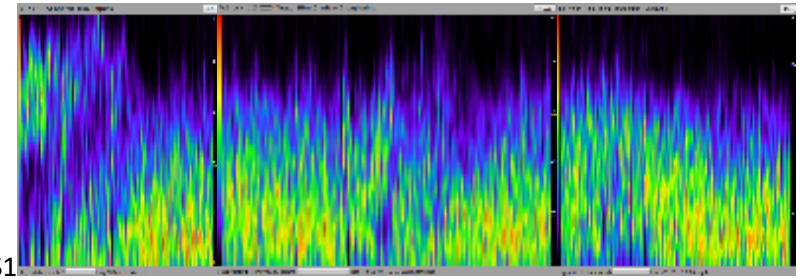
S2 

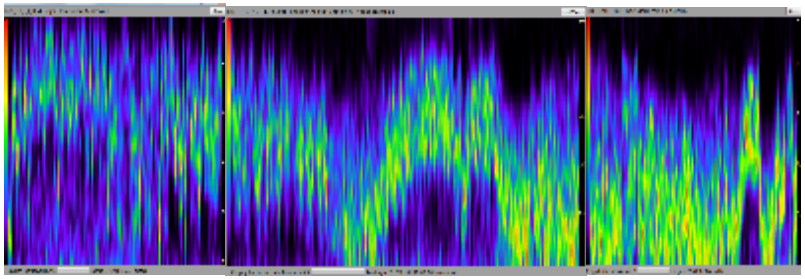
S17 

S18 

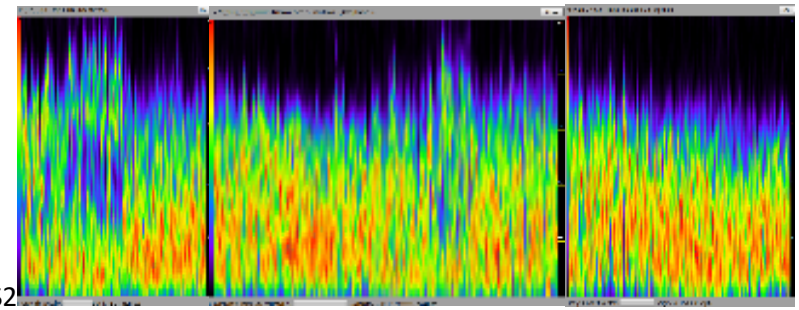
Date of monitoring: 19-21 Dec 16 - 9-11 Jan 17

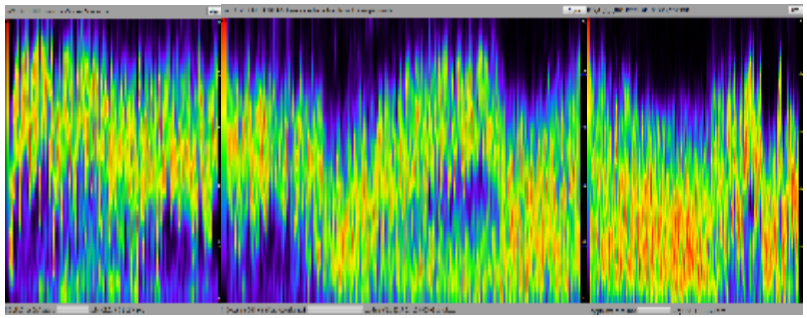
S1



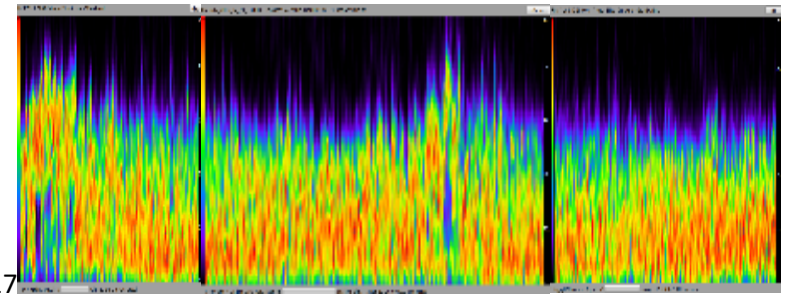


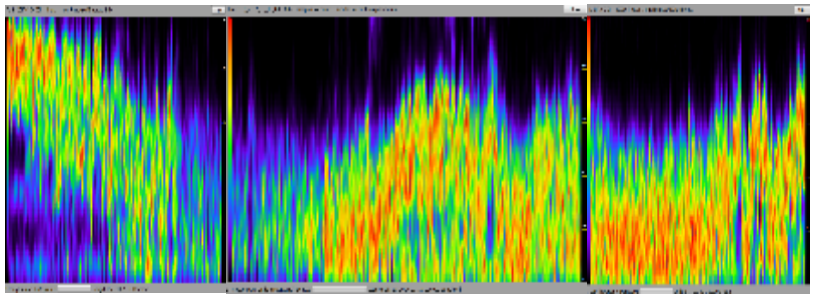
S2



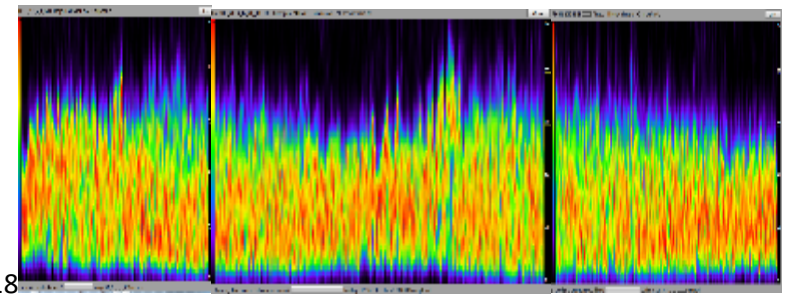


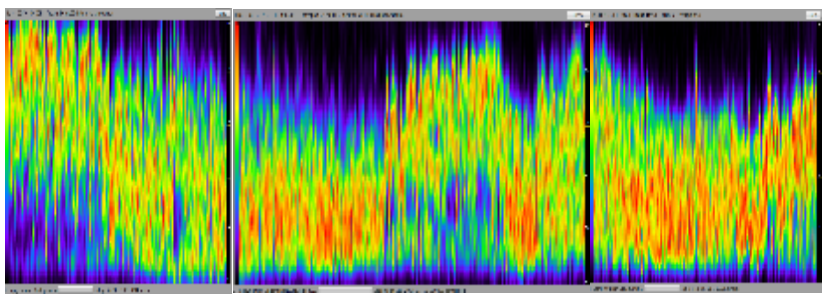
S17



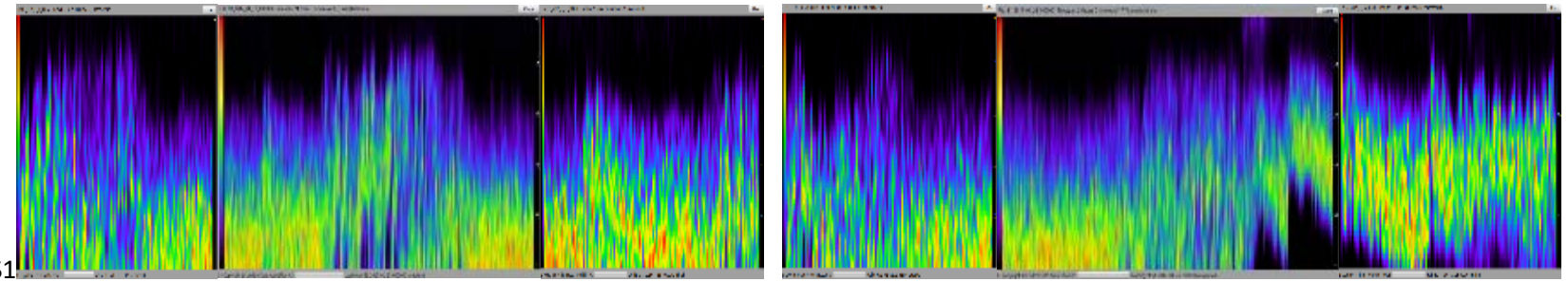


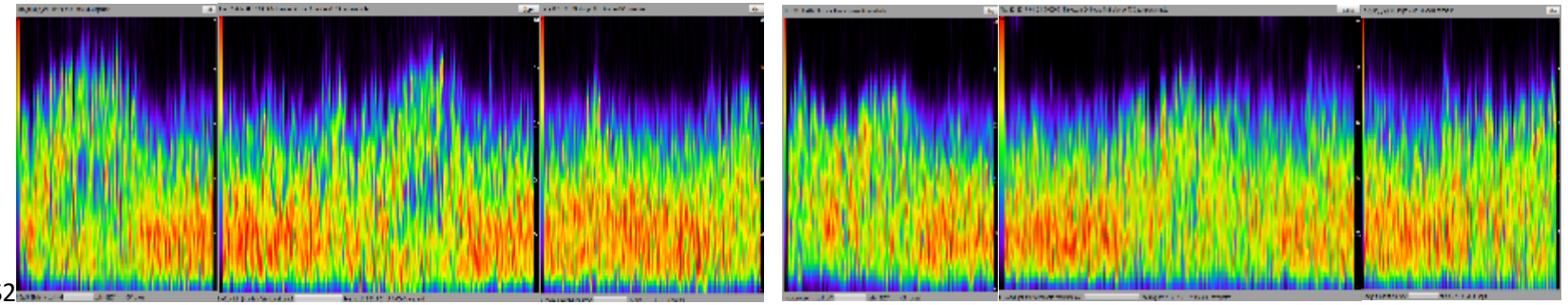
S18

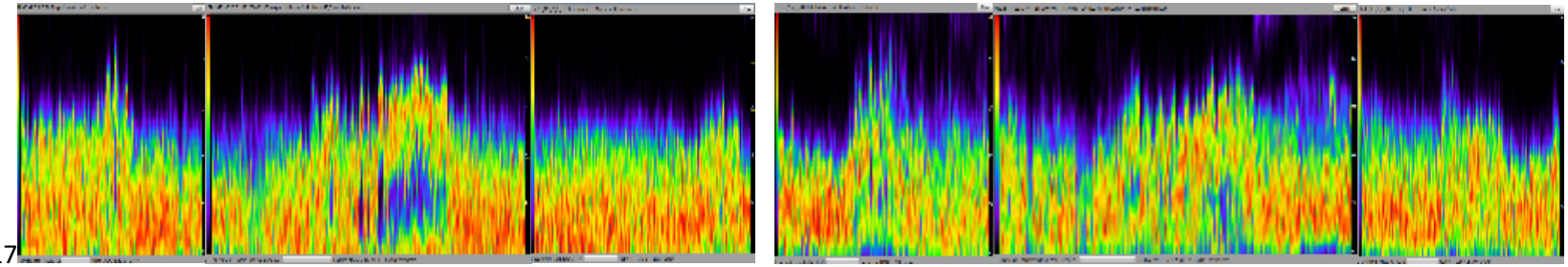


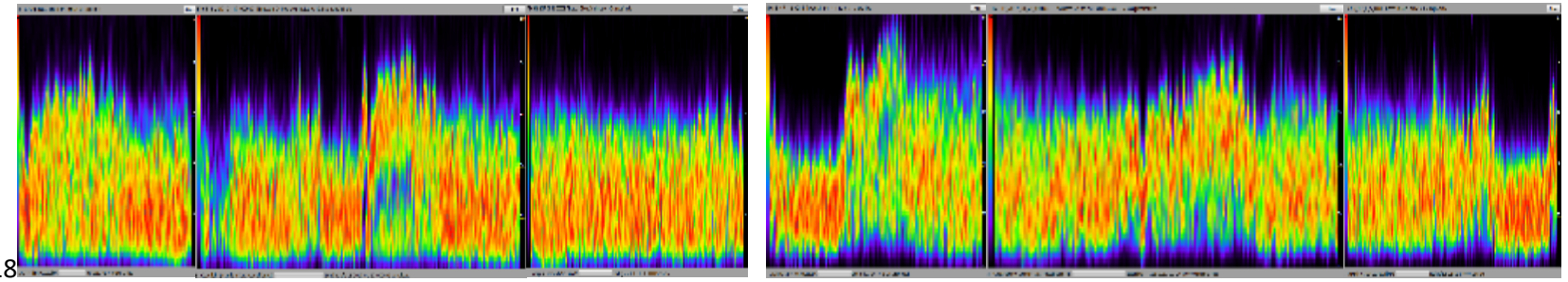


Date of monitoring: 16-18; 23-25 Jan 17

S1 

S2 

S17 

S18 

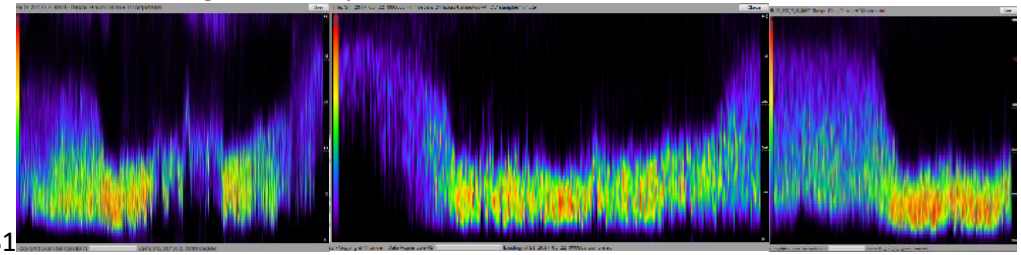
APPENDIX 2

Echographs from the experimental cages during the rial in the Souda cage farm.

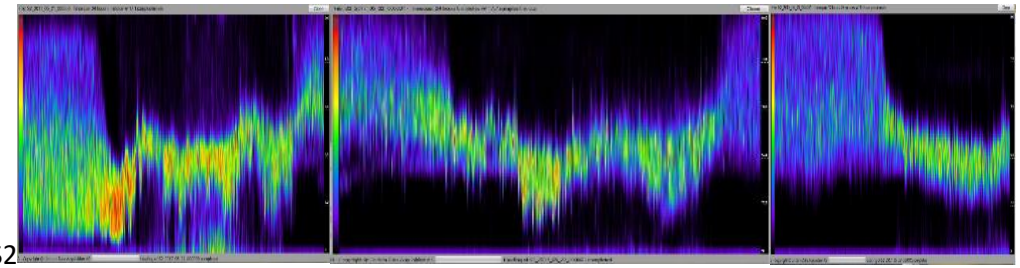
Cages noted as S1 and S2 were the ones fed normally while cages noted as S17 and S18 were the ones fed with submerged distribution.

Date of monitoring: 21-23 May 17

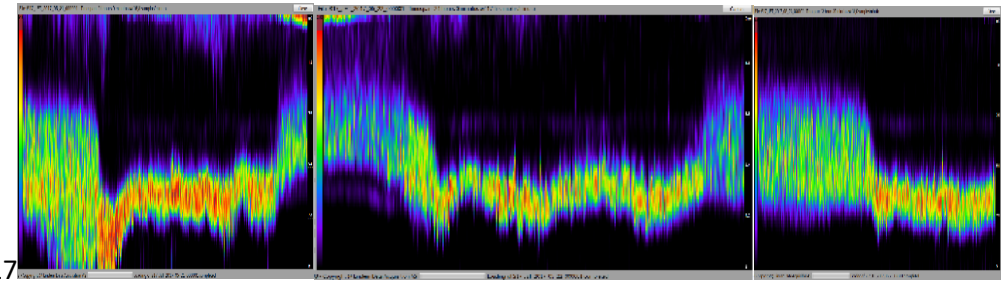
S1



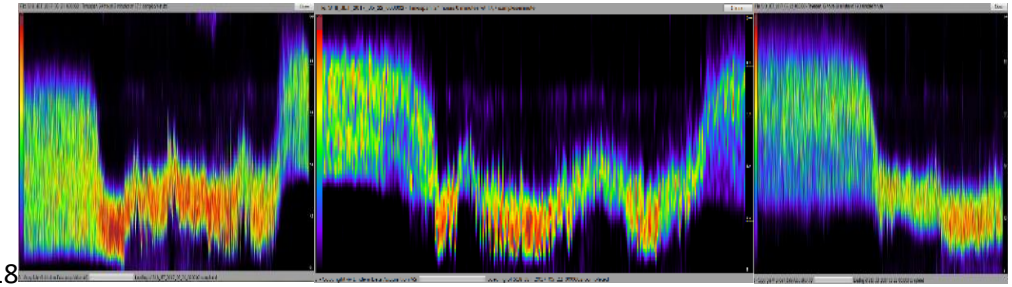
S2



S17

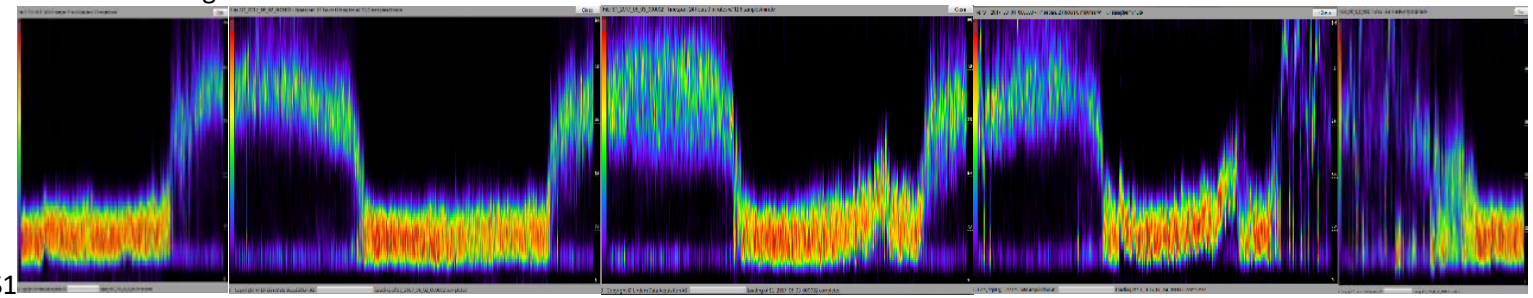


S18

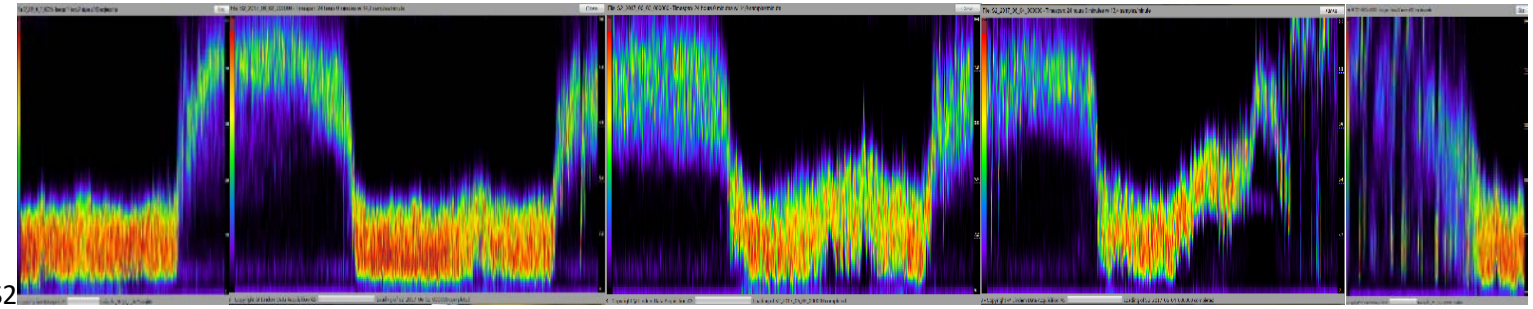


Date of monitoring: 01-05 Jun 17

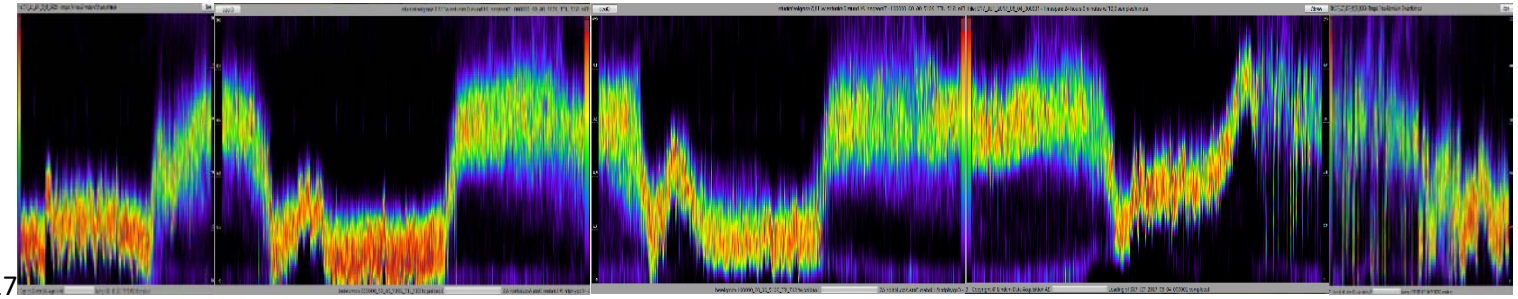
S1



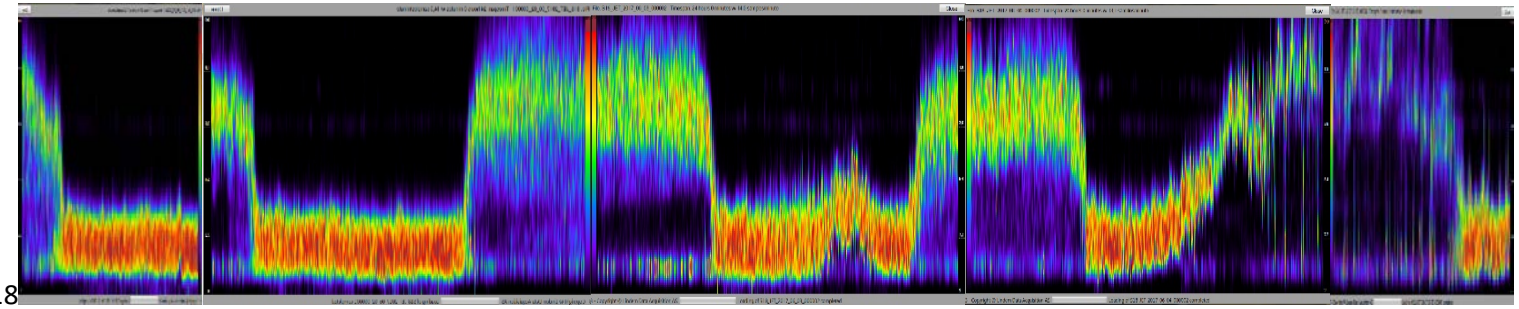
S2



S17

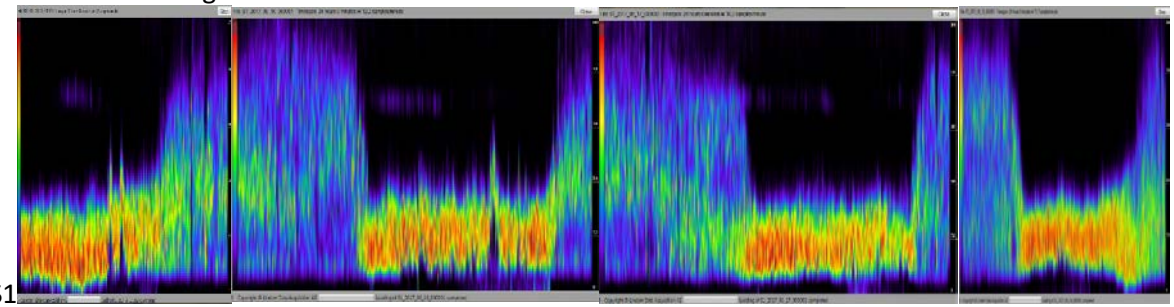


S18

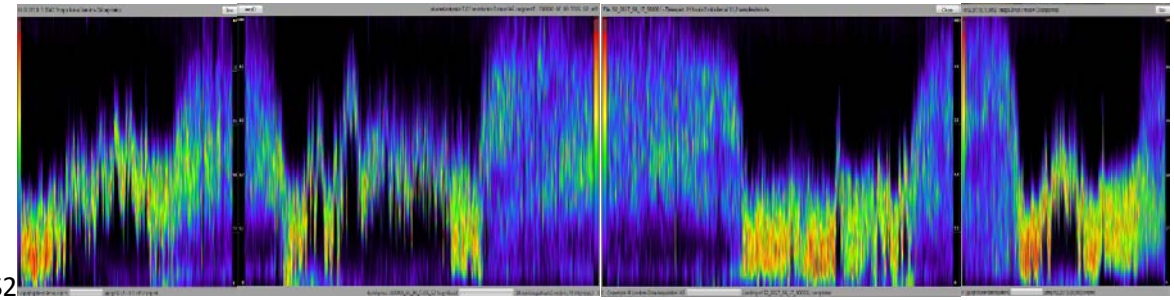


Date of monitoring: 15-18 Jun 17

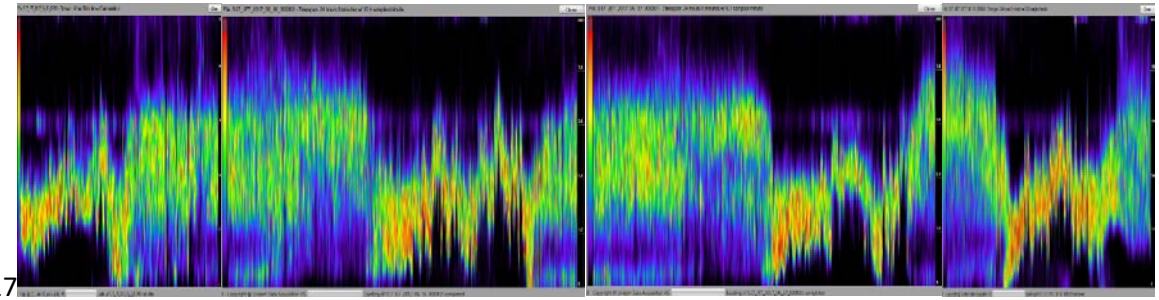
S1



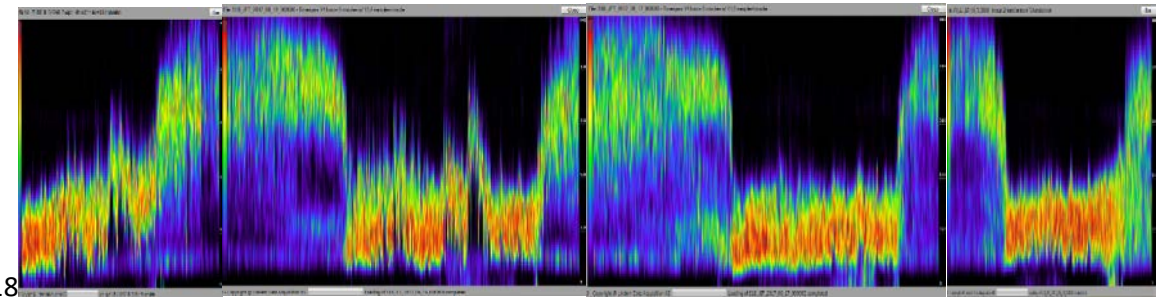
S2



S17

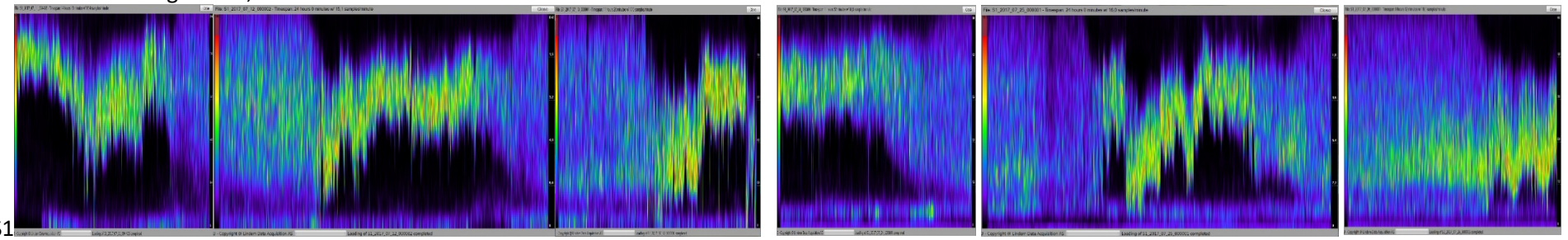


S18

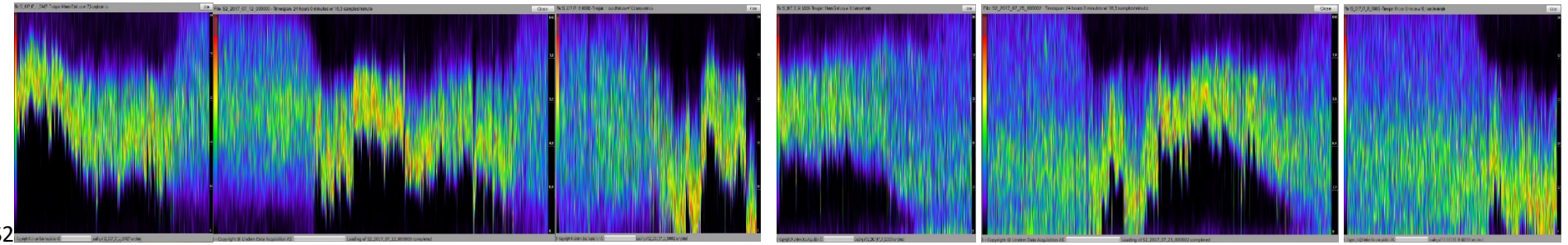


Date of monitoring: 11-13; 24-26 Jul 17

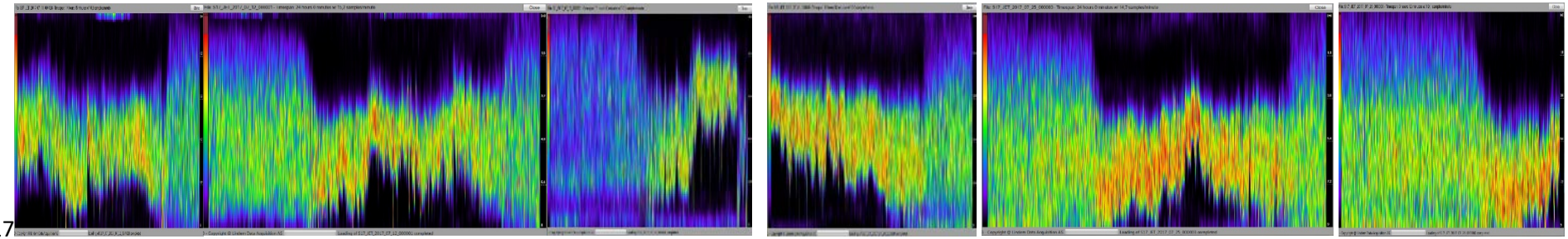
S1



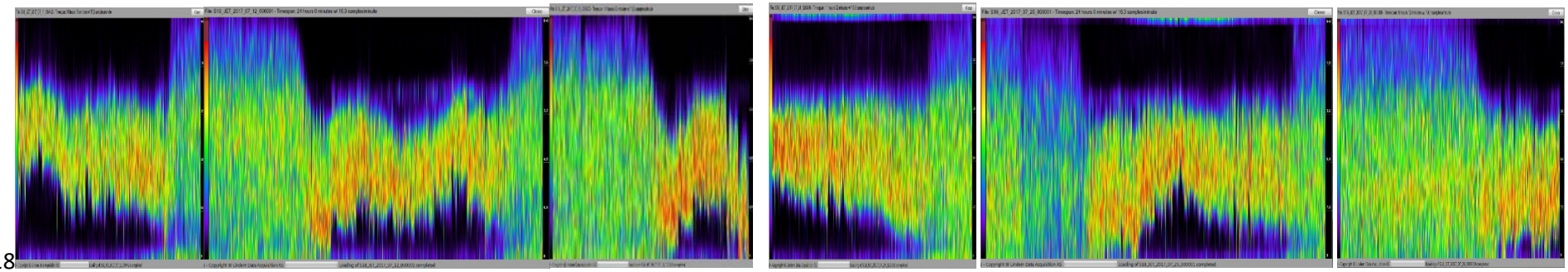
S2



S17

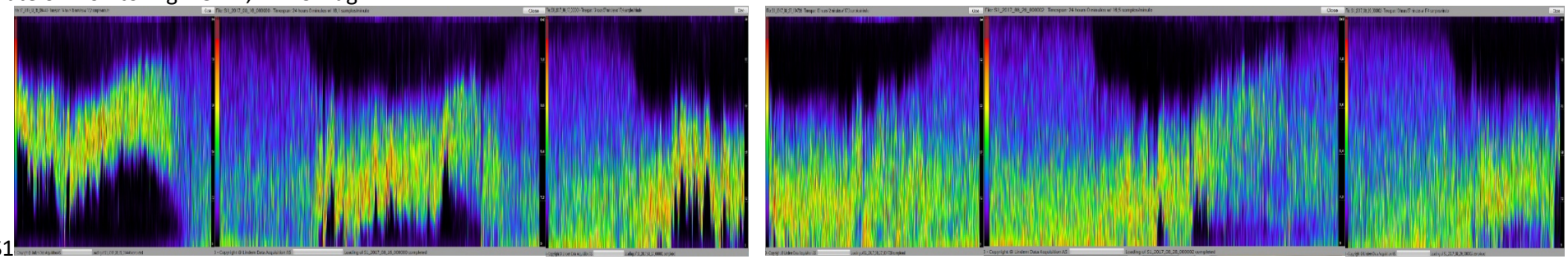


S18

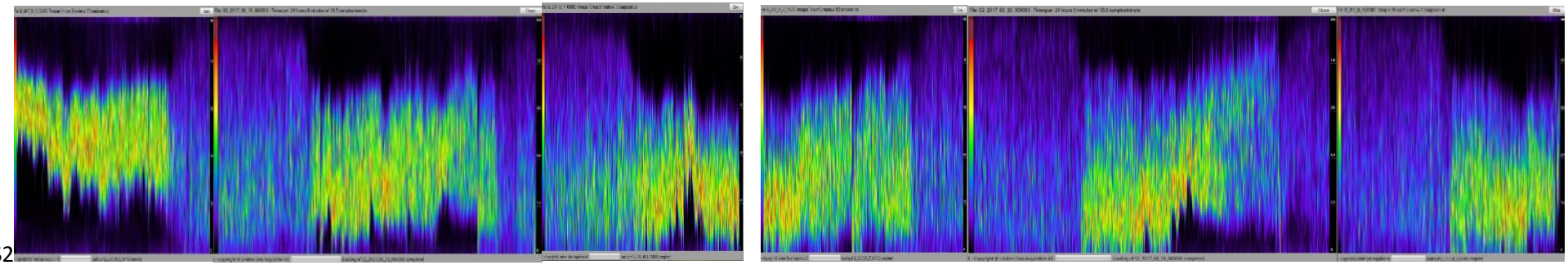


Date of monitoring: 15-17; 27-29 Aug 17

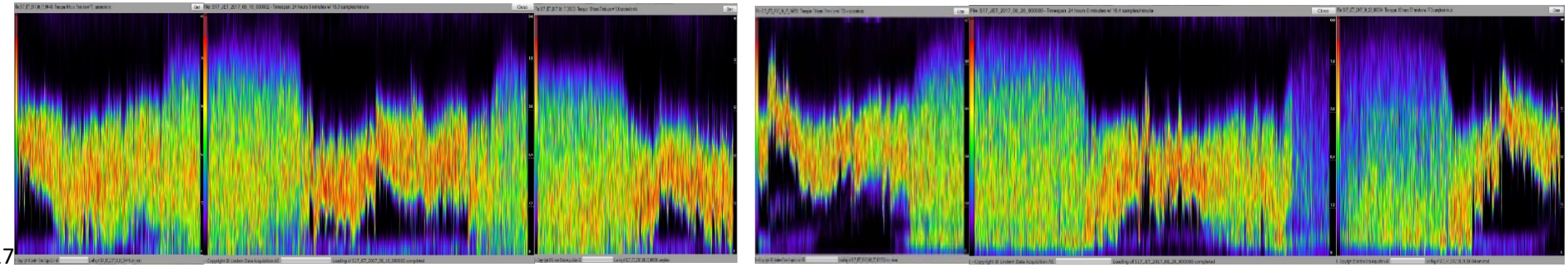
S1



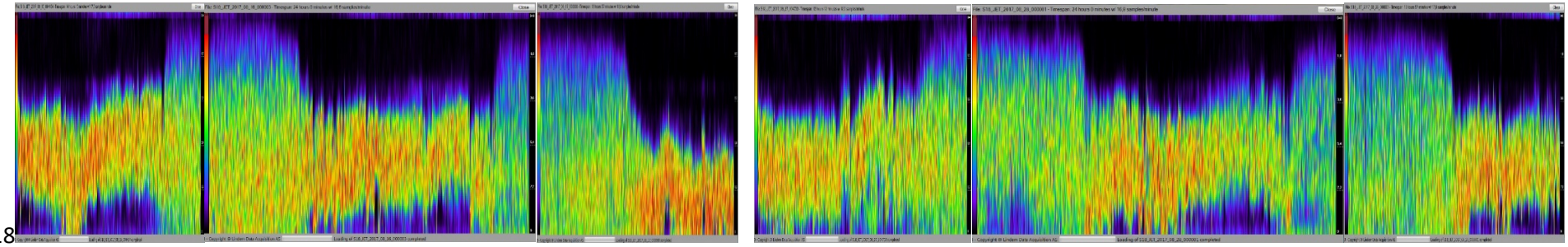
S2



S17

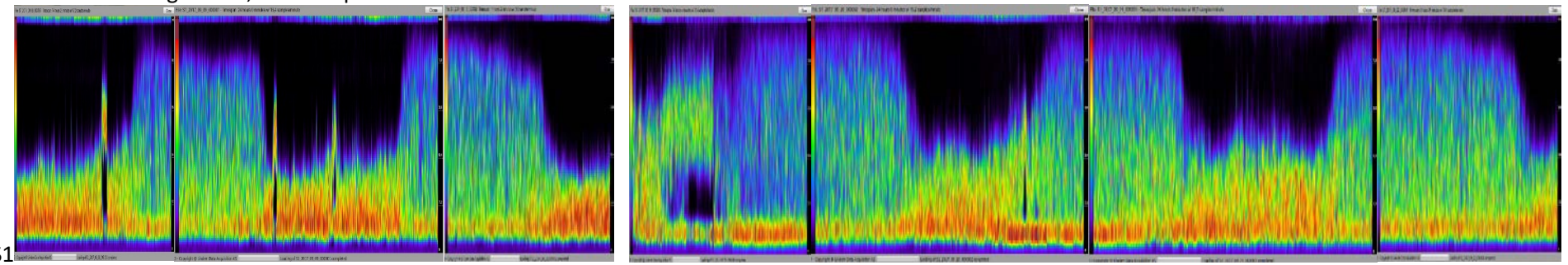


S18

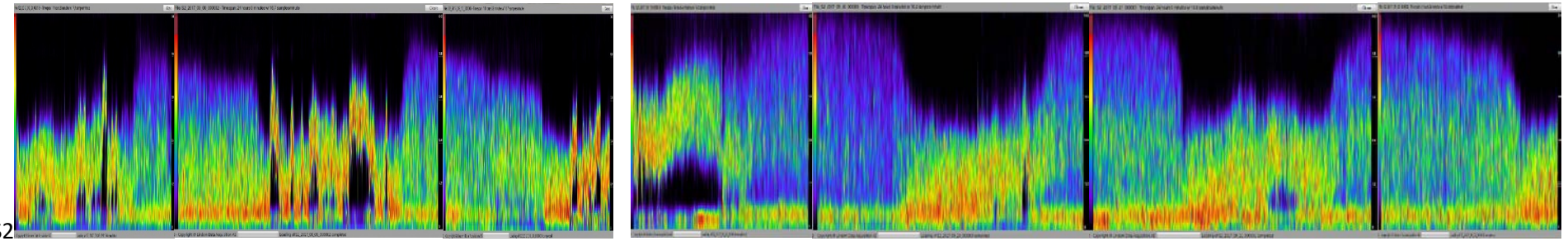


Date of monitoring: 08-10; 19-22 Sep 17

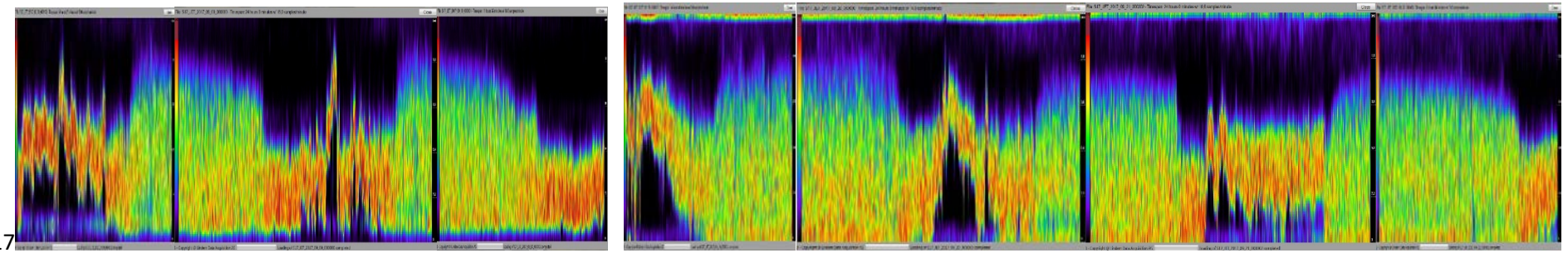
S1



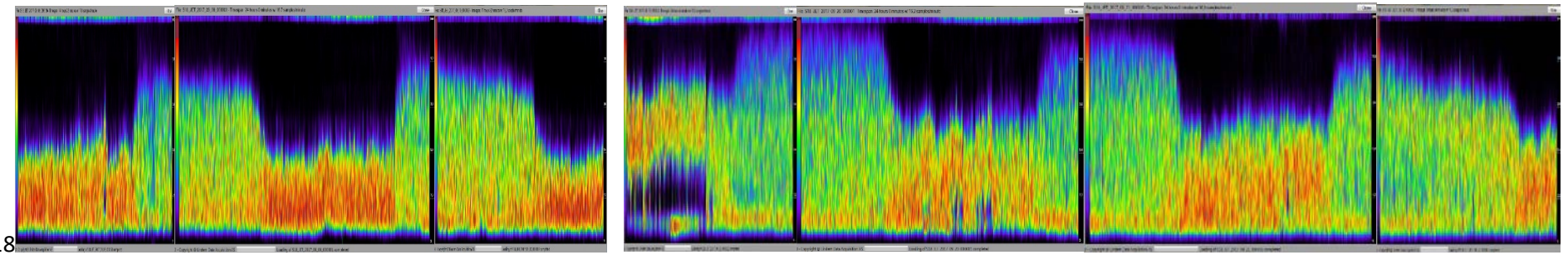
S2



S17

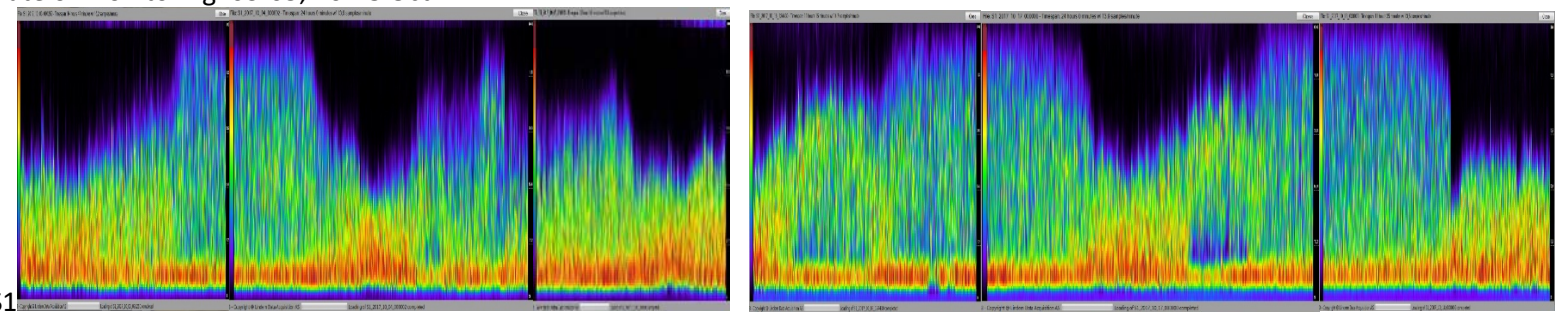


S18

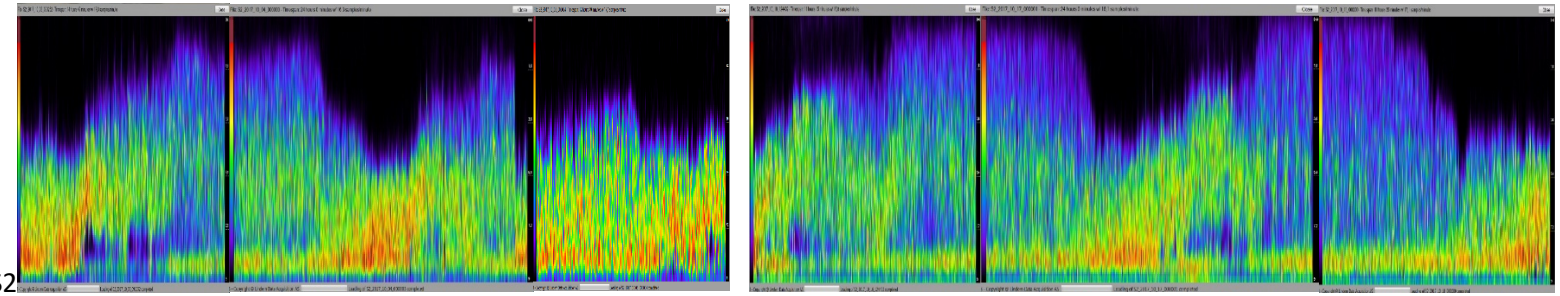


Date of monitoring: 03-05; 16-18 Oct 17

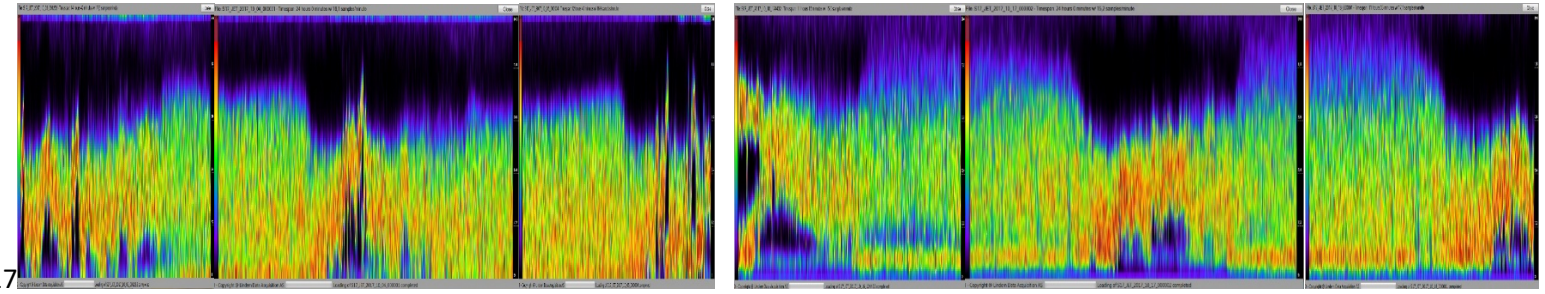
S1



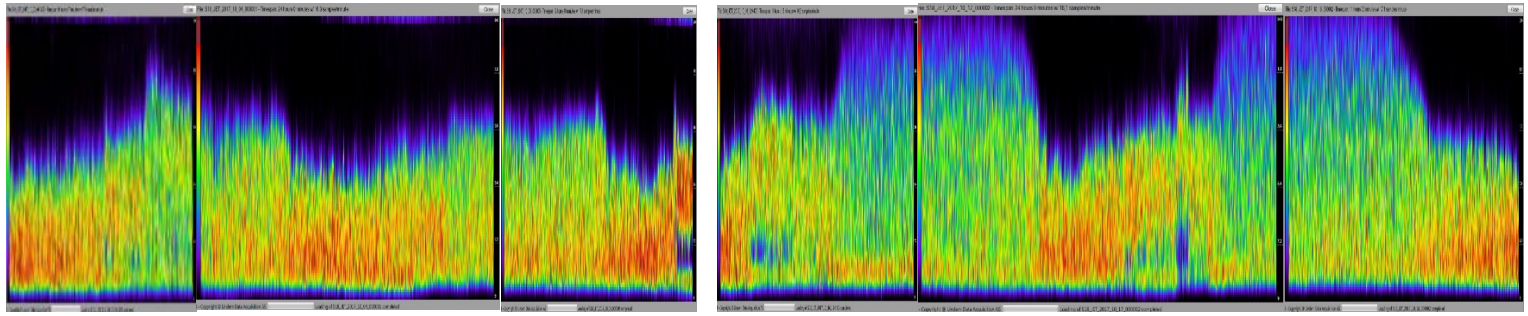
S2



S17

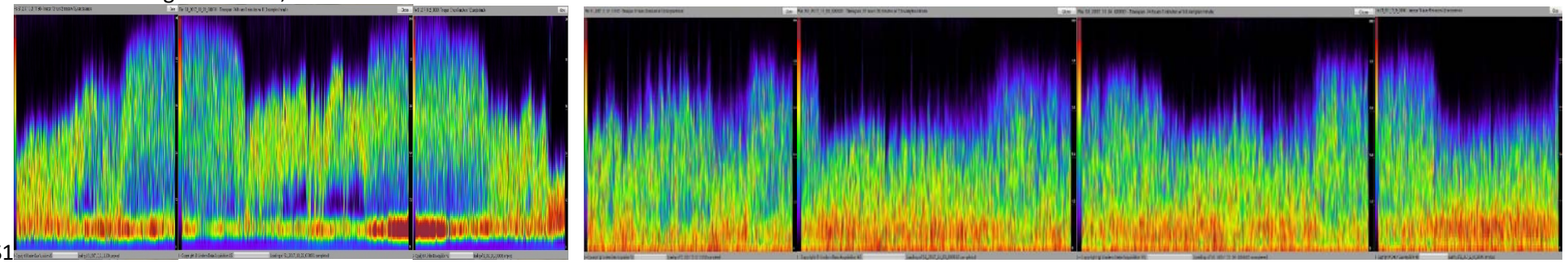


S18

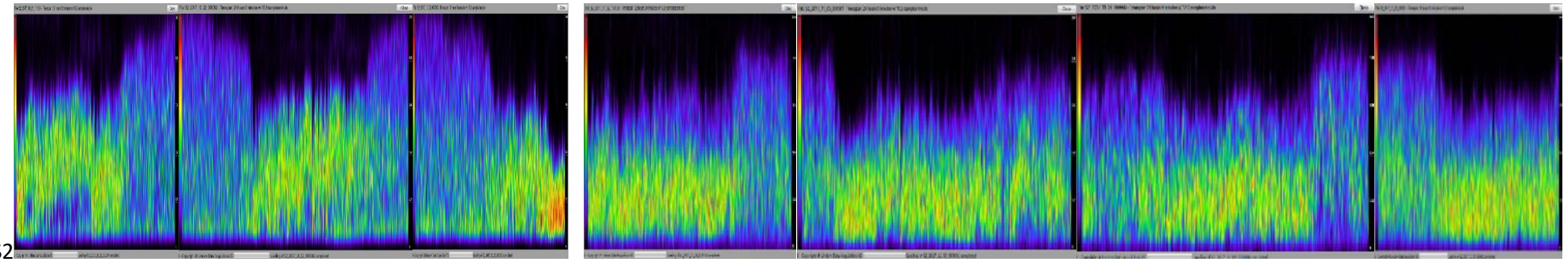


Date of monitoring: 21-23 Oct; 02-05 Nov 17

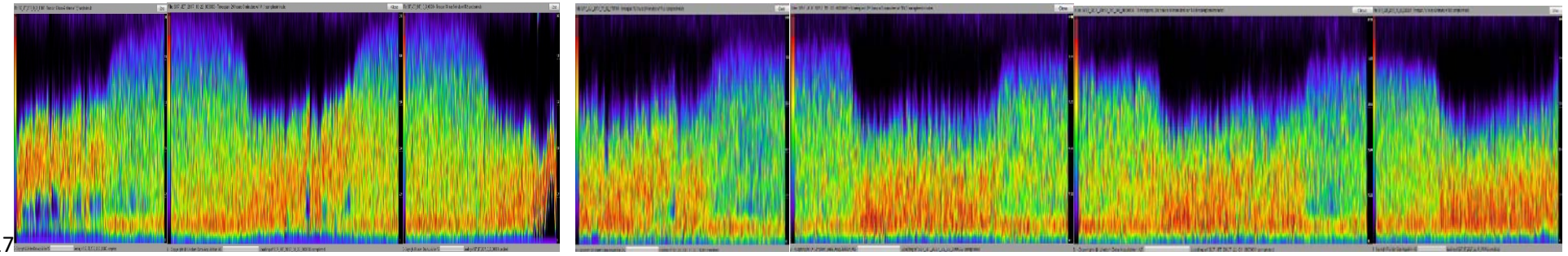
S1



S2



S17



S18