TECHNICAL REPORT

Effects of offshore and onshore aquaculture ambient noise on *Sparus aurata* juveniles

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Contents

ABSTRACT 5
1. INTRODUCTION AND BACKGROUND 6
1.1 Objectives 9
2. MATERIALS AND METHODS 10
2.1. Acoustic equipment and recording/projection procedures 14
2.2. Blood sampling procedures and body measurements 18
2.3. Biochemical and haematological analytical methods 20
2.4. Statistical analysis 22
3. RESULTS AND DISCUSSION 23
4. REFERENCES 31

List of Figures

Figure 1. Facilities of IAMC-CNr of Capo Granitola 10
Figure 2. Concrete tanks of "AcquaAzzurra" FishFarm in Pachino, Sicily - Italy 10
Figure 3. Transporting tank
Figure 4. Experimental fiberglass tanks 12
Figure 5. Schematic view of the Experimental Design 13
Figure 6. Equipment for the acoustic data aquisition 14
Figure 7. One-second spectogram of the ambient experimental tank noise and of different noise stimuli 15
Figure 8. Recording the acoustic stimuli inside an experimental tank 17
Figure 9. Audio projection desk station 17
Figure 10. Underwater loudspeaker 18
Figure 11. Sea bream placed in the stunning tank 19
Figure 12. Body measurement equipment 20
Figure 12. Blood collection procedure 20
Figure 14. Contaglobuli automatico Heco Vet C 21
Figure 15. Weights and Lengths results 24
Figure 16. Weight and Length Curve results. 25
Figure 17. Haematological parameters results 27
Figure 18. ROMs, TACs , A/G Ratio, Lysozyme and Total Proteins values 28
Figure 19. Number of dead fishes 29

ABSTRACT

The present study evaluated the impact of sea background noise (the acoustic environment of the offshore aquaculture system) and onshore aquaculture system's ambient noise on welfare of gilthead sea bream juveniles (*Sparus aurata*). In particular primary, secondary (biochemical and haematological indexes) and tertiary (growth performances) stress responses were evaluated. The experiment lasted 120 days during which two different playlists of acoustic stimuli were projected inside six experimental tanks (each condition was replicated in three tanks). Offshore aquaculture noise conditions were recreated as the typical acoustic field in proximity of an offshore sea cage for fish farming using a random sequence of quiet sea background and boat noises. The acoustic field inside an onshore open concrete tank for fish farming represented the onshore aquaculture noise conditions. The other three tanks were used as a control condition without acoustic projection. The weights and lengths of fish exposed to offshore aquaculture noise were higher than the specimens in the control and onshore aquaculture groups. Moreover, higher levels of serum cortisol, glucose, red blood cell count, haematocrit value and haemoglobin content and lower levels of white blood cells were recorded in fish groups from the control and onshore treatments. Reactive Oxygen Metabolites (ROMs) and Total Antioxidant Capacity (TACs) of fish exposed to the boat acoustic condition after 40 days showed a significant increase compared to those of fish of control group. After 80 days lower values of TACs were recorded in the boat acoustic condition compared to control and on-shore aquaculture acoustic condition.

These results allow us to hypothesise that offshore aquaculture noise and the sea soundscape in particular positively influence growth performance and could reduce stress and improve the welfare of the sea bream.

1. INTRODUCTION AND BACKGROUND

Aquaculture has the potential to play a major role in feeding the human population in the future with a growth rate between 1970 and 2006 of 6.9 per cent per annum (FAO 2009). Marine finfish aquaculture in Europe is dominated by two major species, Atlantic salmon (*Salmo salar*) in the north, with an annual production of almost 900,000 t (EC Fisheries, 2011), and gilthead sea bream (*Sparus aurata*) in the south, with an estimated production (2008) of almost 129,000 t y^{-1} (FEAP, 2009). In both cases, as well as for species such as the European sea bass *Dicentrarchus labrax*, which is cultivated in smaller quantities, the market acceptance of the cultivated product is high (Verbeke et al., 2007), and wild-captured fish are often available only at premium prices that are inaccessible to most consumers.

Aquaculture systems comprise different typology of farming structures that are included into two larger categories: the onshore and offshore farming system. Although onshore aquaculture corresponds to a very significant proportion of world aquaculture, due to the role of Asia Pacific region in global production, an important development is currently occurring in Europe and North America, driven by the increased interest in offshore aquaculture, made possible through improvements in culture structures (Aguilar-Manjarrez et al., 2008). There are a number of potential benefits in placing culture structures such as sea cages some distance from the shore, reducing visual impacts (Byron and Costa-Pierce, 2010; Byron et al., 2011), and promoting greater dispersion of waste products and uneaten food, by taking advantage of stronger hydrodynamics and greater water column depth (Holmer, 2010). Fish welfare in offshore farms is expected to improve due to higher water quality (Pelegri et al., 2006) with less influence from terrestrial run-off and coastal activities, and waste products from farming are rapidly diluted, reducing the local environmental effects and increasing the carrying capacity of the farming sites (Holmer, 2010).

On this regard, recently there is an increasing focus on both the welfare and ethical treatment of aquaculture finfish (Ashley, 2007; Conte, 2004). The potential sources of stress in aquaculture facilities can be many and varied, the effects of which are often amplified and may lead to poor welfare and compromise the health conditions of farmed fish, thus affecting also the profitability of the aquaculture industry (Ellis et al., 2002; Conte, 2004; Huntingford et al., 2006).

Although stressors in an aquaculture setting are unavoidable (Ashley, 2007), the fundamental goal for successful growth and production is the development of optimal strategies and practices that effectively manage or mitigate acute and/or chronic stressors (Craven et al., 2009).

It is well known that fish are often subjected to adverse stimuli that cause acute (Pickering 1981; Pottinger *et al.* 1999) or chronic (Pickering 1981; Montero *et al.* 1999) stress. External

(environment) and internal (disease, metabolic unbalance) stressors can determine significant modifications of some biochemical parameters (Heath, 1990) that could be able to reveal a poor welfare status of animals. Stress stimuli induce fish to react with a primary neuroendocrine response, represented by an increase in corticosteroids (in particular with a cortisol levels perturbation) and catecholamines (Pickering 1981). As a direct consequence of their high levels in the circulatory system, a wide range of secondary stress responses can be observed, such as the increase in blood glucose (Pickering 1981; Melotti *et al.* 1992) from tissue reserves of glycogen. Moreover, during the adaptive stress response, the haemopoietic activity of the spleen increases, which encourages the production of red blood cells for oxygen transport (Franklin *et al.* 1993) and increase of other correlated parameters such as haematocrit value and haemoglobin content (Pickering 1981; Buscaino et al., 2010; Hady Kacem et al., 1986). Stressful conditions negatively affect also both specific and nonspecific immunity, making fish more susceptible to disease (Pickering and Pottinger 1989). On this regard, the oxidative stress depicts the existence of products called free radicals (molecules possessing an unpaired electron) and reactive oxygen species (ROS), which are formed in normal physiology but become deleterious when not being quenched by a cascade of antioxidants systems. Antioxidant defences aimed to protected cells and tissues from oxidative damages and neutralize the toxicity of ROS (Asagba et al., 2008). When ROS production is increased the disturbed balance between oxidant and antioxidant factors results in a prooxidative condition.

Oxidative stress is an important component of the stress response in marine organisms, which are exposed to a wide variety of environmental stressors on varying temporal and spatial scales (e.g. temperature variations, ultra-violet radiation, anthropogenic contamination). The activation of immune cells can be a source of the stress-induced ROS production and antioxidant enzymes in immune cells play an important role in preventing the ROS-induced injury (Babior, 2000). Oxidante antioxidant balance is critical for immune cell functions because of its protective effect of the maintenance of cell membrane integrity and functionality (Knight, 2000; Celada and Nathan, 1994). Immune cells are particularly sensitive to oxidative stress because (1) their membranes contain high concentrations of polyunsaturated fatty acids that are very susceptible to peroxidation, and (2) they produce large amounts of ROS when stimulated. Moreover, membrane-related functions are critical in maintaining normal function of immune cells and their ability to defend against foreign antigens (Yuli et al., 1982).

Until now are still unknown the potential different influence on fish physiology and welfare of specific acoustic background field in onshore and offshore aquaculture system. Sound in an important means of communication in aquatic environments because it can be propagated rapidly

(five times faster than in air) and it is not attenuated as quickly as other signals such as light or chemicals (Smith et al 2004). During the last 50 years, the increase of anthropogenic activities led to a considerable increase in the ambient noise (Hildebrand 2009, Ross 2005) that altered the "soundscape" on a global scale and the current estimate is that the noise in the oceans due to shipping is increasing at about 0.4 dB per year (Ross, 2005). The noise pollution produced by the maritime traffic is characterized by signals that cover a wide range of frequencies. The signals generated mainly by container ships, ferry boats, boats for recreational activity, fishing boats and research vessels are focused around the low frequencies. Other acoustic signals generated at low and high frequencies are produced by the equipment used by ships, fishermen, oil industry, oceanographers, geologists, meteorologists. For example, the sonar used by fishermen (sonar) became an essential tool for searching fish schools. Other measuring instruments are the air guns used for geophysical sampling.

Fish are exposed to a wide range of ambient noise in onshore and offshore culture conditions. In offshore cage condition, fish are exposed to noise generated by cage machinery, marine traffic of different typologies of boat and sea background noise. An even great amount of noise from multiple sources is generated in onshore aquaculture systems due to the use of aerators, air and water pumps, tractors, harvesters, water circulations, feeding and sounds that originate from the activities of personnel managing the facility (Bart et al., 2001).

Recently, there is an increased interest on the effects of anthropogenic noise on marine fish (Popper, 2003). Several field and laboratory studies evaluating the effects of sound on fish have shown that increased ambient sound levels could alter their habitat selection, behaviour, and ecology (Pearson et al., 1992; Knudsen et al., 1994; Engås et al., 1996; Sand et al., 2000; Tolimieri et al., 2002; Popper, 2003). Noise pollution can cause negative effects on fish physiology and welfare such as reduced growth rates (Sun et al., 2001), hearing damage (Codarin et al., 2009; Enger, 1981; Hastings et al., 1996; Sverdrup et al., 1994; Scholik and Yan, 2001; Amoser and Ladich, 2003; McCauley et al., 2003) and stress response (Bart et al., 2001; Engas et al., 1996; Myrberg, 1980; Popper et al., 2005; Smith et al., 2004; Wysocki et al., 2006). Smith et al. (2004) examined the short- and long-term effects of increased ambient sound on the stress and hearing of goldfish (*Carassius auratus*) recording significant threshold shifts in hearing that increased linearly up to approximately 28 dB after 24 h of noise exposure and transient spikes in plasma cortisol levels. Moreover, Santulli et al. (1999) observed variations of cortisol, glucose, lactate, AMP, ADP, ATP and cAMP (typical primary and secondary stress parameters) in different tissues of *Dicentrarchus labrax* exposed to air gun detonations. Buscaino et al. (2010) showed a disturbance effect of a short-

term noise exposition (0.1–1 kHz linear sweep, 150 dBrms re 1 μPa) on motility, glucose, lactate and haematocrit levels of sea bream and sea bass.

However, only few studies have investigated the effects of noise on fish physiology, growth, and survival within fish culture systems. Terhune et al. (1990) observed decreased growth rates of Atlantic salmon (*Salmo salar*), in fiberglass tanks with sound levels 2–10 dB re 1 μPa higher at 100–500 Hz than concrete tanks. Recently, Wysocki et al. (2007) found that the hearing, growth, survival, and disease resistance of rainbow trout (*Oncorhynchus mykiss*), cultured within noisy recycle systems, were not negatively impacted by long-term exposure to intensive aquaculture production noise (115, 130, and 150 dB re 1 μPa rms). Recently, Davidson et al. (2009) observed no significant differences in mean weight, length, specific growth rates, condition factor, feed conversion and survival of rainbow trout (*Oncorhynchus mykiss*) exposed for five months to noise treatments (117 and 149 dB re 1 μPa rms) recorded in an intensive recycle aquaculture system.

1.1 Objectives

The present study investigated the effects of ambient noise in onshore and offshore aquaculture systems on various welfare indexes in gilthead sea bream juveniles (*Sparus aurata*). Fish welfare was evaluated by estimating primary, secondary (assessments of selected biochemical and haematological parameters) and tertiary (growth performances) stress indexes after 40, 80 and 120 days of exposure to the two different ambient noise conditions. The biochemical parameters included serum cortisol, blood glucose levels, Total Antioxidant Capacity (TAC), Reactive Oxygen Metabolites (ROMs), Total Protein, Albumine, Globuline, A/G ratio, lysozyme and antiprotease activity, and the haematological parameters included white blood cell count (WBC), red blood cell count (RBC), haematocrit value (PCV) and haemoglobin concentration (Hgb). Growth was evaluated through body weight and length measurements.

2. MATERIALS AND METHODS

The present study was carried out at the Institute for Marine and Coastal Environment of the National Research Council (CNR-IAMC) facilities of Capo Granitola (SW Sicily) (see Figure 1) for 120 days from May to September 2012.

Figure 1. Facilities of IAMC-CNR, Detached Unit of Capo Granitola

Approximately 400 gilthead sea bream (*Sparus aurata*) juveniles of four months of age were retrieved from the aquaculture AcquaAzzurra fish farm in Siracusa (SE Sicily) (Fig. 2).

Figure 2. Concrete tanks of "AcquaAzzurra" FishFarm in Pachino, Sicily - Italy

Fish were captured and placed into a transferring tank (total capacity of 500 litres) (Fig. 3) added with a constant oxygen supply form a cylinder. The transport by car lasted five hours.

Figure 3. Transporting tank of 500 litres in capacity

After transport, the fishes were transferred to a circular PVC tank (5.0-m diameter and 1.5-m depth) for a two-month acclimation period.

270 fish were randomly sorted from the holding tank, individually weighed and measured (14.70 ± 4.7 g in weight and 9.75 ± 0.96 cm in fork length; mean ± SD) and assigned to nine identical experimental square fiberglass tanks (Fig. 4) in groups of 30 individuals.

Experimental fiberglass tanks (side length of 1 x 1 m and depth of 1.5 m). The tanks were equipped with an independent flow-through seawater system from a common source (25 ± 3.7 l min^{-1}; mean ± SD). Salinity was 36.4 ± 0.81 ppt (mean ± SD), and temperature was 20.1 ± 0.78°C (mean ± SD). Sea bream were fed with commercial pellets (Saipa s.r.l. – Macerata, Italy) twice daily.

Figure 4. Experimental fiberglass tanks (side length of 1X1 m and depth of 1.5 m). The tanks were equipped with an independent flow-through seawater system from a common source

Three different acoustic conditions were reproduced in the experimental tanks (each condition was replicated in three tanks):

Offshore aquaculture condition (OFF) - underwater loudspeakers recreated the typical acoustic field in proximity to an offshore fish farm sea cage using a random sequence of sea background and boat noises;
Onshore aquaculture condition (ON) - underwater loudspeakers reproduced the acoustic field inside of an onshore fish farm open concrete tank;
Control condition - without any loudspeakers, fish were exposed to the low-level noise of the experimental tank environment. Within each control tank, a loudspeaker PVC mimic was also set up to maintain the same landscape of the treatment tanks.

A schematic view of the experimental tanks and acoustic stimuli projection equipment is represented in Figure 5.

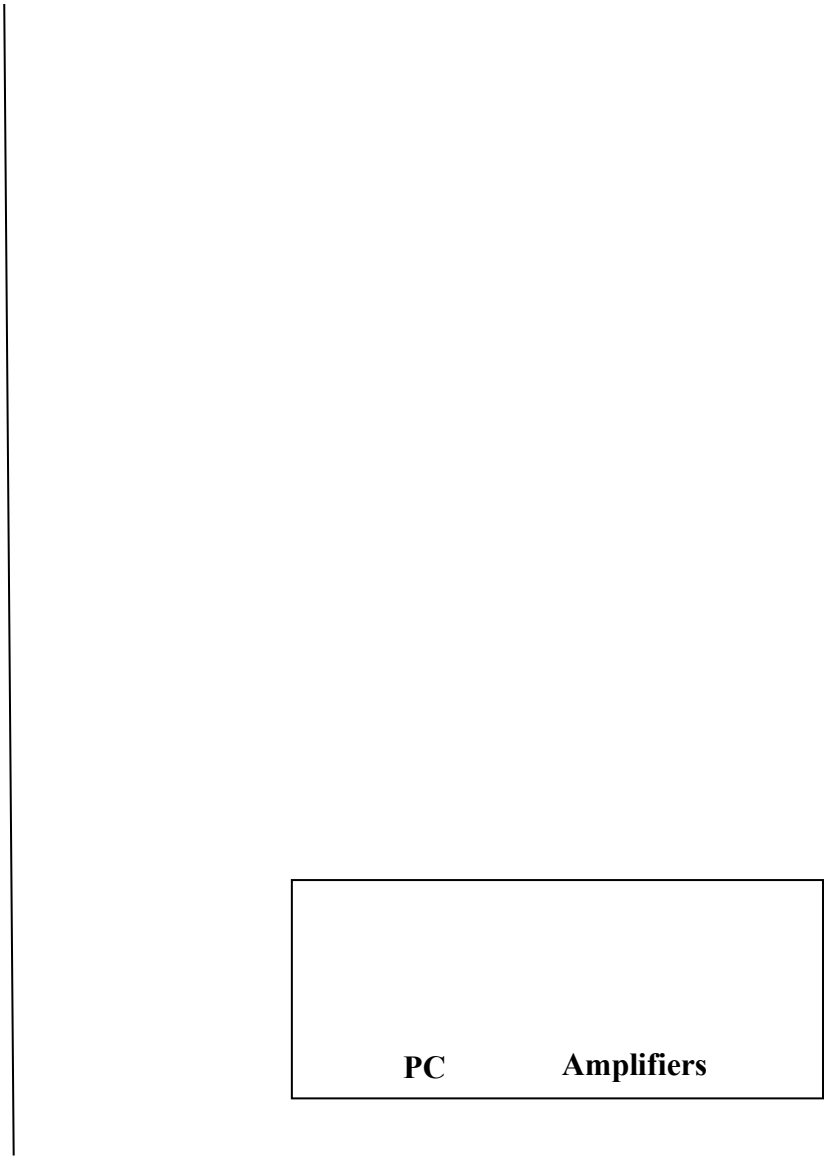

Figure 5. Schematic view of the Experimental Design and real disposition of the three typologies of tanks

During the study period, ten fish per tank were captured and sampled for body measurements (weight and total length) and blood collection for the biochemical and haematological parameter determinations at intervals of 40, 80 and 120 days. At the same time, the number of deceased fish was also registered for each acoustic exposure treatment

2.1. Acoustic equipment and recording/projection procedures

To obtain acoustic recordings of the noises from onshore and offshore aquaculture conditions and the tank environment, a calibrated hydrophone (model 8104, Bruel & Kjer) was used with a sensitivity of −205.6 dB re 1 V/µPa ± 4.0 dB in the 0.1-Hz to 80-kHz frequency band. The hydrophone was used with a preamplifier (VP1000, Reson) with a 1-MHz bandwidth single-ended voltage, with the high-pass filter set at 10 Hz and a 32-dB gain. The equipment was connected to a digital acquisition card (USGH416HB, Avisoft Bioacoustics, set with no gain) managed by Avisoft Recorder USGH software (Avisoft Bioacoustics) (Fig. 6). The signals were acquired at 300 kilosamples s^{-1} at 16 bits and analysed by the Avisoft-SASLab Pro software (Avisoft Bioacoustics). The format of file was .wav.

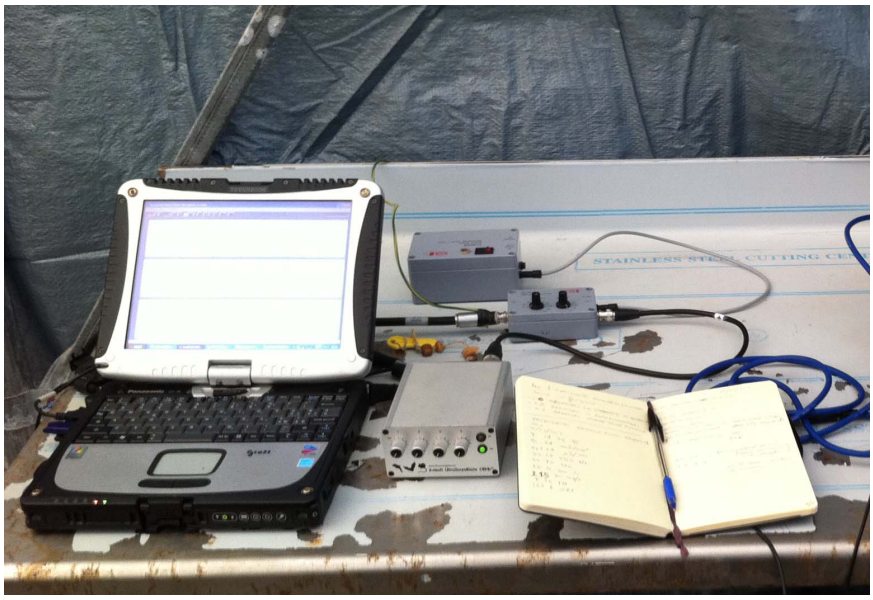

Figure 6. Equipment for the acoustic data aquisition

The ON noise was recorded inside three concrete rectangular tanks (2.55 x 13 m and 2 m deep) at the Acqua Azzurra fish farm.

The OFF noise was recorded at sea in different locations where gilthead sea bream typically inhabits to obtain a verisimilar background acoustic condition of a generic offshore aquaculture farm, where the dominant noise is represented by the natural sea soundscape (i.e., no passage of boats within a range of 8 kilometres) alternating with the passages of different types of boats (Table 1 and Fig. 7).

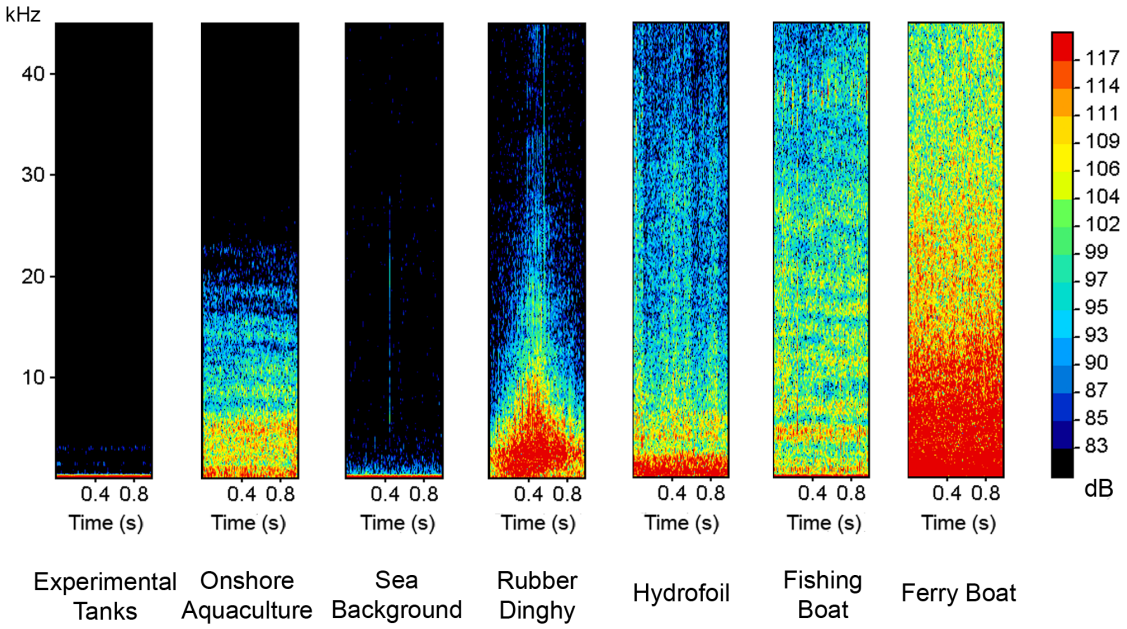

Figure 7. One-second spectogram of the ambient experimental tank noise and of different noise stimuli: frequency (kHz) vs. time (s). The intensity is reflected by the colour scale (dB re 1 µParms, 1024-sample FlatTop window)

The noise in the experimental tanks was recorded to characterise the baseline noise of the study environment.

To project the two acoustic stimuli of the obtained files, ON and OFF noises, inside the experimental tanks, two different playlists (Table 1) were created.

Table 1. Acoustic energy and number of reproduction of the noise stimuli in the two playlists

Noise typology	Description	Duration (s)	Mean total Energy per sec (V^2 s)	Total number of file reproduction in the playlist	Total energy of file or playlist (V^2 s)
ON noise (playlist 1)	Sequence of different noise from aquaculture concrete tanks	90	0.32	50	1430
Total playlist 1		4500			1433
OFF noise (playlist 2)	Quite sea noise background file	30	0.14	140	588.0
	Fishing boat file	46	0.36	1	16.4
	Ferry boat file	101	6.48	1	654.9
	Rubber dinghy file	90	1.62	1	146.2
	Hydrofoil file	63	0.39	1	24.7
Total playlist 2		4500			1430

The acoustic stimuli were recorded inside an experimental tank (Fig. 8) in order to measure the acoustic energy between the two playlists using the "energy of marked section" function in the Avisoft-SASLab Pro software (Avisoft Bioacoustics). First, we evaluated the sum of the total energy as the integral of the squared amplitudes, expressed in volts, multiplied by the duration of the ON playlist. In order to obtain an energy equilibrium between the two playlists, the OFF playlist was created as a mix of repetitions of the background sea-noise file (lower energy file) and boat files with the same amount of acoustic energy as the ON playlist.

Figure 8. Recording the acoustic stimuli inside an experimental tank with the aim to measure the acoustic energy between the two playlists

The two playlists were projected using the "loop mode" function of the Avisoft-SASLab recorder software (Avisoft Bioacoustics) that can randomise the emission files sequence, maintaining the emission ratio between the different files. Each of the two stereo outputs of the PC, corresponding to each playlist, was connected to a Channel Low Impedance Amplifier (model QD-4240 – Inter M, Seoul, Korea) (Fig. 9). Each amplifier was connected to six underwater loudspeakers (Model UW30, Lubell, Columbus, Ohio, USA) (Fig. 10).

Figure 9. Audio projection desk station

Figure 10. Underwater loudspeaker fixed to the support inside an experimental tank

2.2. Blood sampling procedures and body measurements

A standardised handling procedure was applied to minimise the stress involved in blood sampling. Blood withdrawal was always performed between 8.00 a.m. and 12.00 a.m., and feeding was stopped 24 hours before blood collection. Sea bream were quickly dip-netted from the tanks in less than 1 min and immediately anesthetised with 2-phenoxyethanol (1:300 v/v) in a 60-litre bucket. Fish reached stage V of anaesthesia (Summerfelt and Smith, 1990) within 2–3 min (Fig. 11).

Figure 11. Sea bream placed in the stunning tank. 2-phenoxyethanol was added allowing fishes the reaching of the stage V of anaesthesia

Immediately after the stunning procedure, sea bream were weighed to the nearest 0.1 g, measured in fork length to the nearest millimetre (Fig. 12) and finally underwent venipuncture for blood collection. Two operators simultaneously collected the blood from the caudal vein using 1-ml syringes with 25 G X 1 $^{1}/_{2}$ needles in less than 2 min for each fish (Fig. 13).

For each fish, blood samples were stored in 2 different types of tubes: microtubes (0.6 ml Miniplast, LP Italiana Spa, Milano) containing ethylenediaminetetraacetic acid (EDTA; ratio = 1.26 mg/0.6 ml) as the anticoagulant agent for the haematological analysis and eppendorf tubes (1 ml Eppendorf, MBL International Corporation, Woburn, MA USA) with no additive after clotting and centrifugation at 3000 rpm for 10 min at 4 °C measurements. Serum samples were split into several aliquots and frozen at -20 °C and −80 °C until further analyses.

After all body measurements and blood collection, all fish were sacrificed using a highly concentrated anaesthetic bath and successively stored at -80°C for any future analyses.

Figure 12. Body measurement equipment

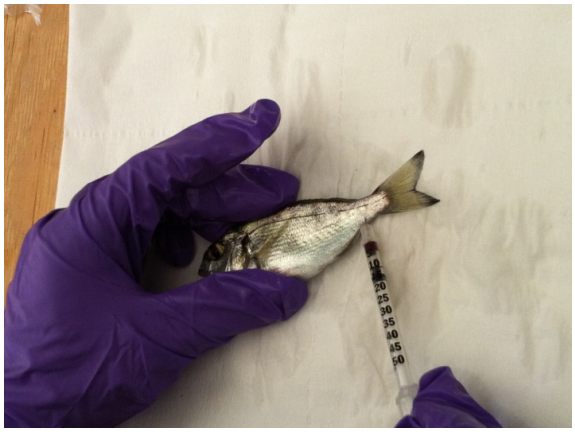

Figure 13. Blood collection procedure

2.3. Biochemical and haematological analytical methods

The immediate assessment of blood glucose concentration was performed using a portable blood glucose analyser (BG STAR, Sanofi Aventis, Milan, Italy).

From serum frozen at -20 °C Cortisol was measured in 25-µl un-extracted serum samples using a commercially available solid-phase ^{125}Iodine radioimmunoassay, Coat-ACount® Cortisol (D.P.C. Los Angeles, CA). Data were obtained with a Kontron Analytical MDA 312 gamma counter and analysed using RIA software. The analytical sensitivity was 2 ng/ml, and the intra- and inter-assay coefficients of variation were 4.7% and 6.4%, respectively.

All blood samples placed in Miniplast microtubes were analysed in duplicate by the same operator immediately after collection. The samples exhibited parallel displacement to the standard curve. The overall intra-assay coefficient of variation was < 5%. Analytical measurements were made to determine the WBC, RBC, PCV and Hgb. These analyses were performed using a blood cell counter already in use in the veterinary field, HeCo Vet C (SEAC, Florence, Italy) (Fig. 14), which was suitably modified by a specific software (SEAC, Florence, Italy) designed for the haematological analysis of fish species following the method adopted by Fazio et al. (2012).

Figure 14. Contaglobuli automatico Heco Vet C

Serum samples frozen at -80 °C were sent to the University of Basilicata laboratories for the measurement of Total Antioxidant Capacity (TAC), Reactive Oxygen Metabolites (ROMs), Total Protein, Albumine, Globuline, A/G ratio, lysozyme and antiprotease activity.

Total Antioxidant Capacity (TAC) was determined using the ferric ion reducing antioxidant power (FRAP) assay as indicated by Benzie and Strain (1996). Firstly, 300 mM sodium acetate buffer, pH 3.6, 10 mM tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 20 mM iron(III) chloride hexahydrate were mixed in a volume ratio of 10:1:1 to generate FRAP fresh daily prepared solution. Subsequently, 10 µL of samples in duplicate were added to 300 µL of FRAP solution in wells of a microtitre plate and the absorbance of the reaction mixture was recorded at 593 nm after 5 min of reaction using a microplate reader (Model 550, BioRad). The standard curve was constructed using iron(II) sulfate heptahydrate at concentrations ranging from 62.5 to 1000 µM (Pearson's correlation coefficient: $r^2 = 1$).

Reactive Oxygen Metabolites (ROMs) were determined using the radical cation N,N-diethyl-*para*-phenylendiamine, (DEPPD) as described by Alberti et al. (2000). Ten µL of samples in duplicate were added to wells of a microtitre plate. Subsequently, 200 µL of a solution containing 0,37 mM DEPPD and 2,8 mM iron (II) sulfate heptahydrate in 100 mM acetate buffer, pH 4.8, was added to each well. After incubation (30 min at 25°C) absorbance was recorded at 530 nm using the microplate reader. The standard curve was constructed using tert-buthyl-hydroperoxide at concentrations ranging from 125 to 1000 µM (Pearson's correlation coefficient: $r^2 = 0.99$).

2.4. Statistical analysis

The Kolmogorov–Smirnov test was used to check the normal distribution of the biometric and biochemical/haematological data.

A one-way analysis of variance (ANOVA) was applied in order to detect significant differences in biometric data (weight and fork length separately) within the experimental tanks, with the factor "tank" (fixed and orthogonal, nine levels).

A two-way ANOVA was applied with two factors (fixed and orthogonal): "time" (3 levels: 40, 80, 120 days) and "noise exposition" (3 levels: ON, OFF, Control), on each set of the biometric and biochemical/haematological data.

Newman-Keuls' multiple comparisons test was used for all *post hoc* comparisons.

P-values of <0.05 were considered statistically significant. All statistical analyses were performed using the STATISTICA 7.0 (StatSoft) software package.

3. RESULTS AND DISCUSSION

All fish used in the present study were healthy as was indicated by their activity and exterior appearance and the bacteriological and parasitological examinations carried out in samples.

Gilthead sea bream weights and lengths at the beginning of the study didn't show statistical difference between specimens of control, offshore and onshore aquaculture condition tanks ($p>0.05$). In the present study, no statistical differences in weight and length were observed between fish of the three experimental tanks of each acoustic condition (inside condition comparisons) during all sampling time points ($p>0.05$). Moreover, as reported in Figure 15, weights and lengths of control and onshore aquaculture fish groups didn't show significant statistical difference ($p>0.05$) during all the experimental procedure. Conversely, a significant difference was observed in growth between the specimens exposed to the three different acoustic field conditions. Post hoc analysis, testing against an $\alpha=0.01$, indicated that the weight and length of fish exposed to offshore aquaculture condition were higher than the specimens of control and onshore aquaculture groups after 40, 80 and 120 days, with statistical differences both for weight and length 80 and 120 days after the beginning of the experimental procedure ($p<0.05$ and $p<0.01$ respectively) (Figure 15).

In particular, at the end of the experimental phase (120 days) fish of offshore aquaculture group showed an increase of 17.26% and 16.95% in weight and of 4.48% and 4.31% % in length respect control and onshore aquaculture groups respectively (see Figure 16). Moreover, fish of offshore aquaculture group increased from 14.79 ± 0.48 g to 26.97 ± 0.87 g (Mean ± SEM) in weight and from 10.69 ± 0.11 to 12.58 ± 0.13 cm (Mean ± SEM) in length with a final growth increment from the beginning of the experiment of 82.35% and 17.68% for weight and length respectively.

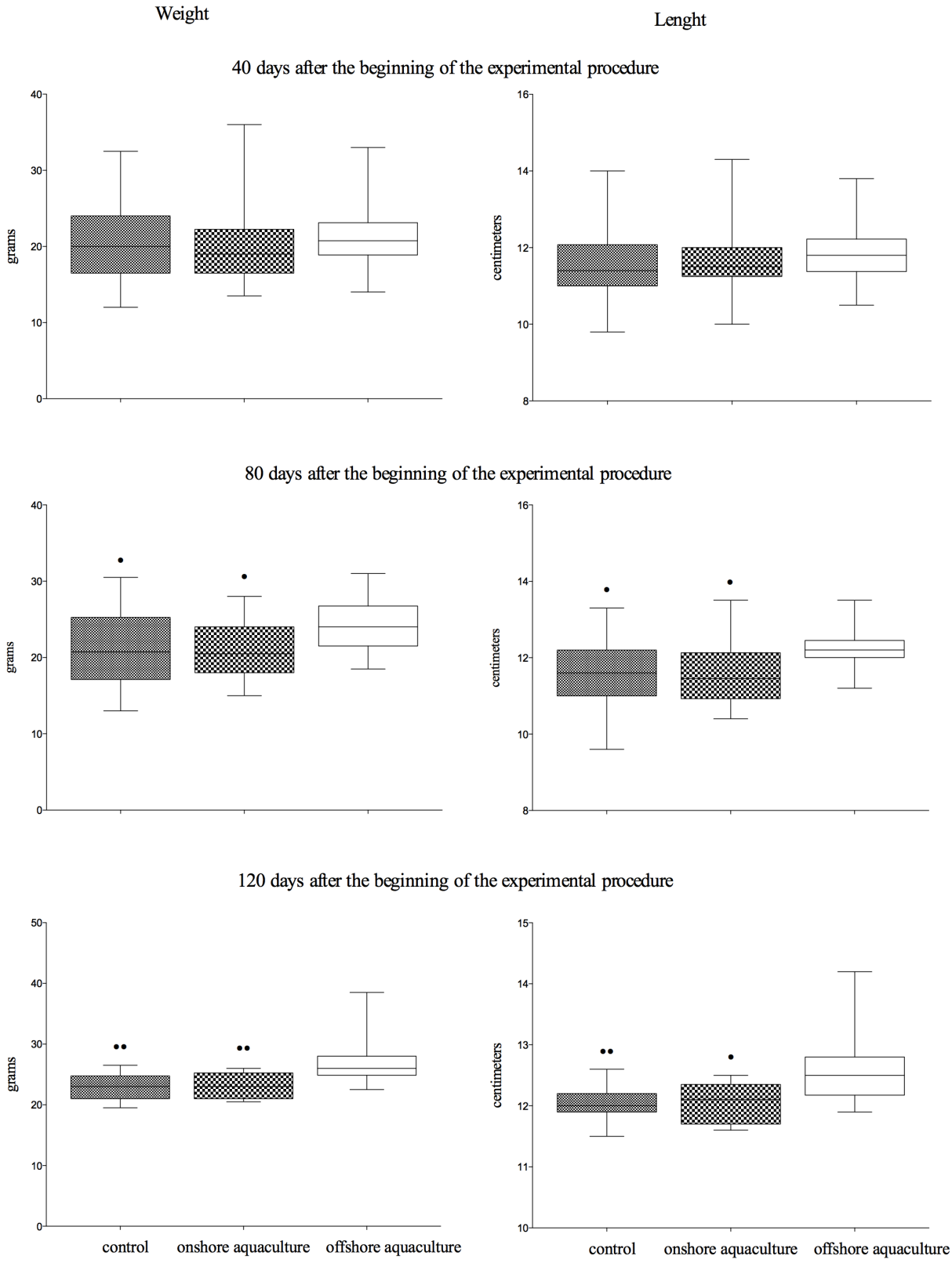

Figure 15. Weights and Lengths (Mean ± SEM) of sea bream from control, onshore and offshore aquaculture groups 40, 80 and 120 days after the beginning of the experimental procedure; onshore aquaculture/control vs. offshore aquaculture •= $p<0.05$; ••=$p<0.01$

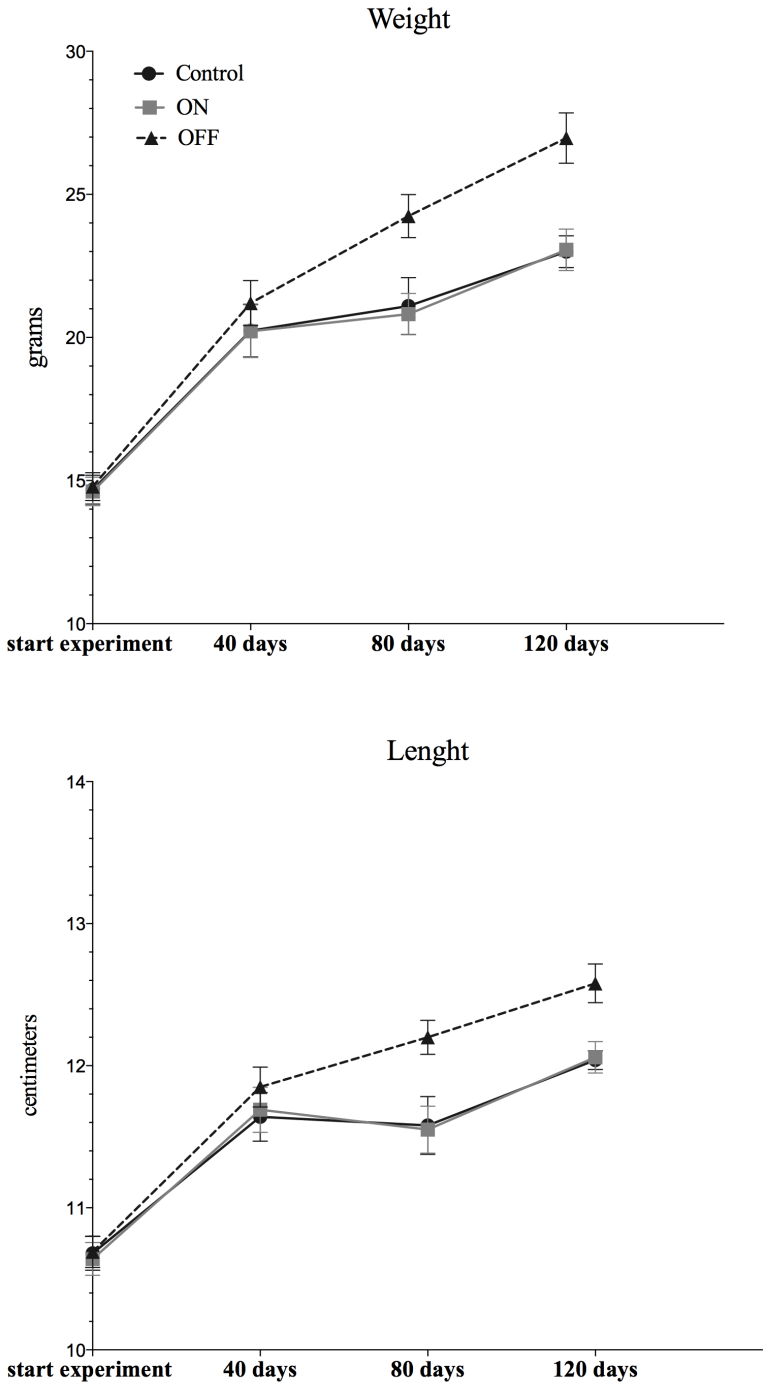

Figure 16. Weight and Length Curve (Mean ± SEM) of control, onshore and offshore aquaculture groups of sea bream from the start to the end of the experiment (after 120 days).

In our study, no statistical differences in all haematological parameters measured were observed among fish of the three experimental tanks of each acoustic condition (inside condition comparisons) during all sampling time points ($p>0.05$).

Cortisol response was higher in sea bream exposed to onshore aquaculture acoustic condition compared to the other groups. Moreover, significantly lower cortisol levels were detected in fish of offshore aquaculture group respect the onshore aquaculture group ($p<0.05$) 40 and 80 days after acoustic exposition and respect control and onshore aquaculture groups ($p<0.05$ and $p<0.01$ respectively) 120 days after the acoustic exposition.

At the first sampling time (40 days post-acoustic condition exposition), statistical differences in glucose levels were observed only between onshore aquaculture and offshore aquaculture fish groups with the latters that showed significant lowest levels ($p<0.05$) (Fig. 17). At 80 and 120 days post-acoustic condition exposition, both control and onshore groups exhibited a significant increase of glucose levels respect fish of offshore aquaculture condition ($p<0.01$).

At the end of the experiment, cortisol and glucose levels increased in all three experimental groups, even if significant differences in cortisol levels between the first (40 days) and final sampling (120 days) point for onshore aquaculture group ($p<0.05$) were recorded, and statistical differences in glucose levels between the first and the second sampling point ($p<0.01$) and between the first and the final sampling point ($p<0.01$) both for control and onshore aquaculture groups were observed.

A significant increase of RBC, PVC e Hgb levels was observed in control and onshore aquaculture groups in comparison to the fish of offshore aquaculture group after 40, 80 and 120 days after the acoustic field expositions ($p<0.05$, $p<0.01$; see Figure 17). No statistical differences between control and onshore aquaculture groups were detected ($p>0.05$) at all sampling points.

WBC levels showed the highest values at the first sampling time in offshore aquaculture group with statistical differences only in comparison to onshore aquaculture group ($p<0.05$). Moreover, significant highest levels of WBC were recorded in offshore aquaculture fish group respect both control and onshore aquaculture groups at 80 and 120 days post-acoustic condition exposition ($p<0.05$, $p<0.01$; see Figure 17).

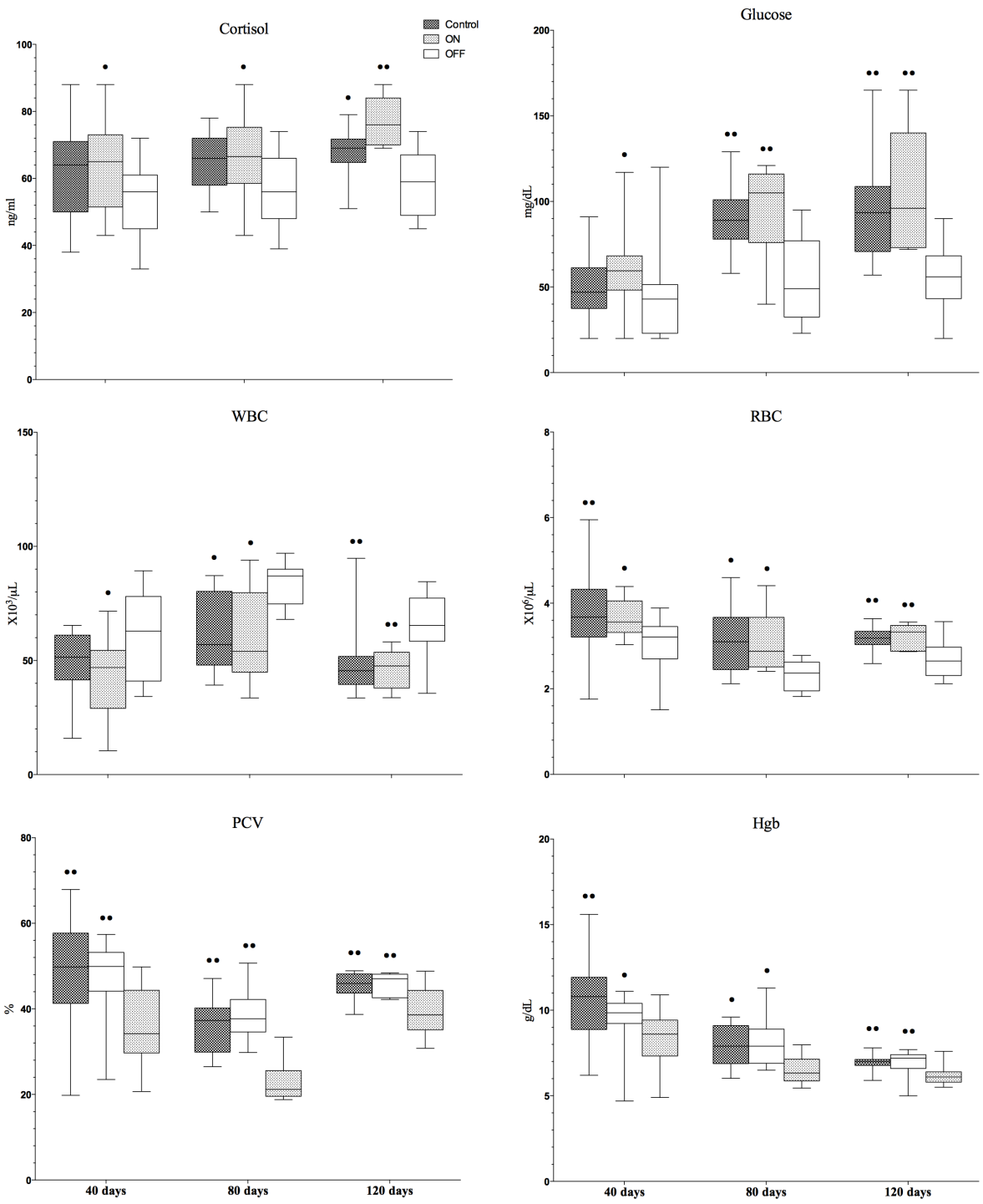

Figure 17. Haematological parameters (Mean ± SEM) of sea bream from control, onshore and offshore aquaculture groups 40, 80 and 120 days after the beginning of the experimental procedure; onshore aquaculture/control vs. offshore aquaculture •= $p<0.05$; ••=$p<0.01$

ROMs of fish exposed to the boat acoustic condition after 40 days showed a significant increase compared to those of fish of control group ($p<0.01$); after 40 days, TACs were significantly higher in fish exposed to the boat acoustic condition compared to control ($p<0.01$), while, after 80 days lower values were recorded in the boat acoustic condition compared to control and on-shore aquaculture acoustic condition ($p<0.01$) as reported in Figure 18. Both ROMs and TACs showed statistically higher levels after 40 days respect 80 days in all the experimental conditions ($p<0.001$) (Fig. 18).

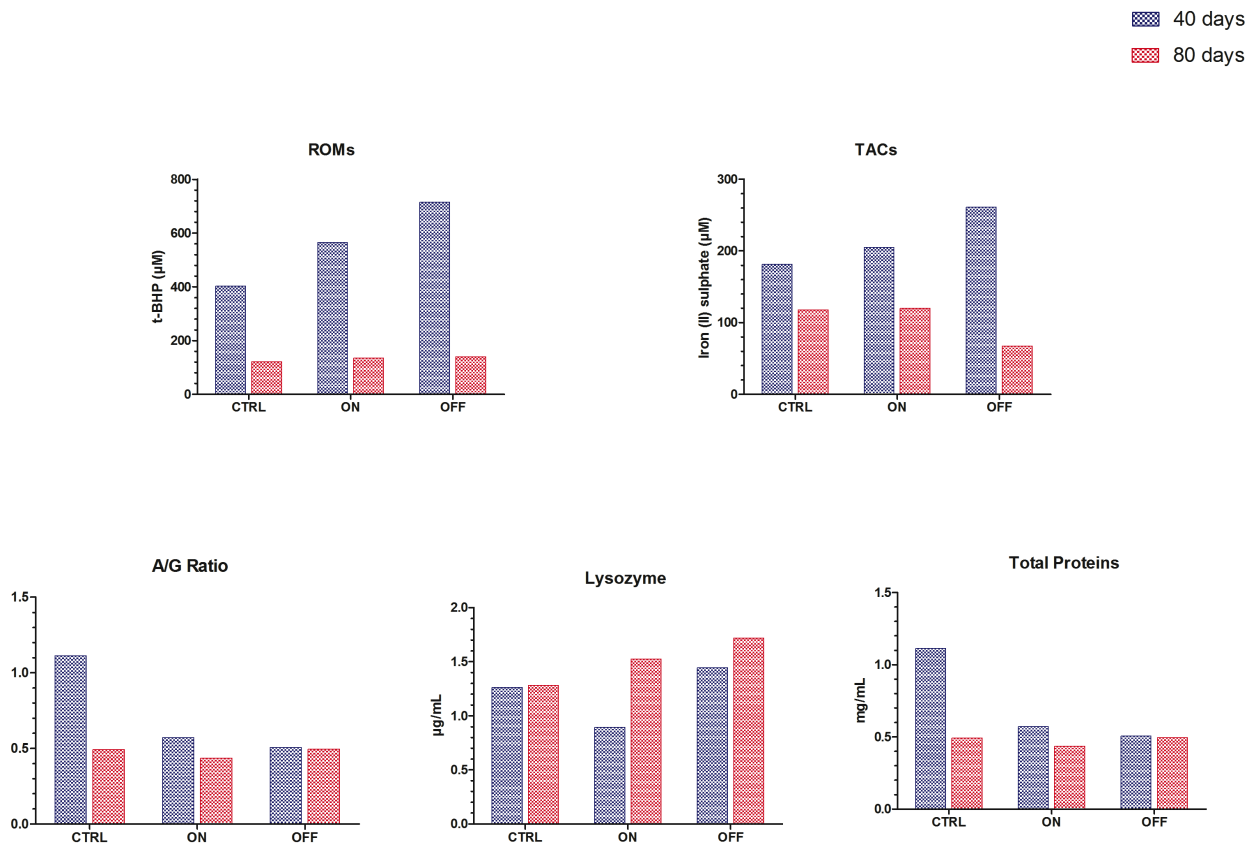

Figure 18. ROMs, TACs , A/G Ratio, Lysozyme and Total Proteins values in the three experimental fish groups

Results on fish mortality are reported in Figure 19. No mortality was observed during the acclimatization period. At the first sampling time (40 days after the beginning of the experiment), the absence of fish mortality was recorded in control and offshore aquaculture groups while only one death was observed in onshore aquaculture group. The number of deaths increased during the experimental acoustic expositions reaching 120 days post-acoustic exposition, the 14.44, 23.33 and 13.33% (percentage on total fish present inside the three tank of each experimental condition from 41st to 120th day after the beginning of experiment) in control, onshore and offshore aquaculture groups respectively.

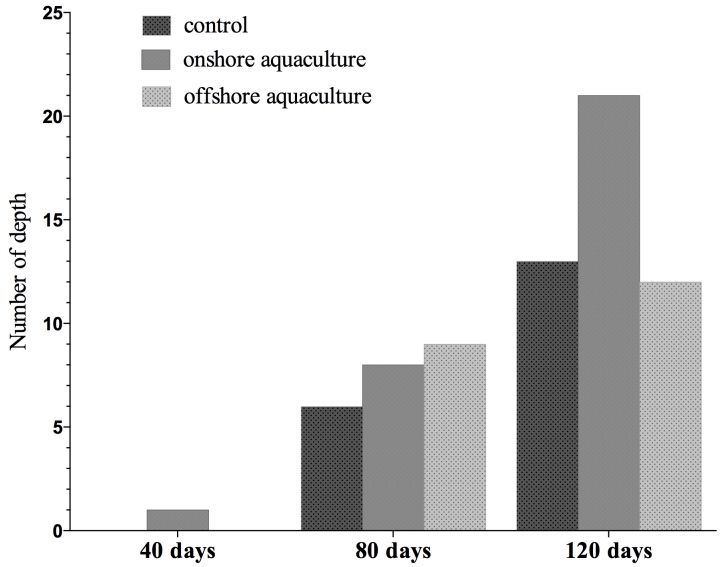

Figure 19. Number of dead fishes from control, onshore and offshore aquaculture groups during the experimental phase

Our results clearly show that the ambient noise affects hardly some biochemical and haematological parameters indexes of acute and chronic stress and the growth rate in gilthead sea bream. In fact, fish exposed to the Offshore aquaculture noise show higher growth performances and changes in the biochemical/haematological responses in comparison to Control and Onshore aquaculture fish groups.

In this experimental test the onshore aquaculture stimulus, as well as the acoustic stimuli from the different type of boat, has the majority of the energy focussed on the frequency range of 0.025-1 kHz. However, we obtained the same results between fish of onshore aquaculture and control groups with the latter that were exposed to lower noise levels respect the other groups. These results indicate a non-effect of onshore aquaculture noise on growth and biochemical/haematological data

collected while a relative positive effect of offshore aquaculture noise. In fact, different effects were observed on fish exposed to offshore aquaculture noise condition where the dominant noise was represented by the sea soundscape alternated by the noise from different kind of boats. Although, previous studies have showed the disturbance effects of boating noise on fish (Codarin et al., 2009; Myrberg, 1980; Sandstrom et al., 2005; Sarà et al., 2007; Wysocki et al., 2006), the different welfare condition observed in the present study could be explained with the very higher number of reproductions of the sea background noise compared to the reproductions of boats noise in the playlist 1. These evidences allow us to hypothesize that the sea soundscape plays a role in the sea bream biology and consequently contributes to improve their welfare status.

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