

Research Article

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Improving aeration for efficient oxygenation in sea bass sea cages. Blood, brain and gill histology

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Abstract: An air diffusion based system (Airx) was developed to control the dissolved oxygen levels in aquaculture sea cages. The system was introduced and then tested for 37 days in a sea bass sea cage (aerated cage). A second sea bass sea cage, without the AirX, was used as a control. Oxygen levels were measured in both cages at the start of the trial, before the AirX system was introduced, and during the working period of the AirX system. Fish samples were collected 15 days after the AirX system was introduced and at the end of the experiment. Blood smears were prepared and examined microscopically. Erythrocyte major axis, minor axis and area of fish erythrocytes were measured. Leucocyte differentiation was also examined. In the control cage, the fish had significantly larger red blood cells when compared with the red blood cells of the fish in the aerated cage. Histological examination of the gills and brain revealed no morphological differences or alterations between the two groups of fish. This study demonstrated that an air diffuser system could improve the water quality of fish farmed in sea cages and enhance sea bass physiological performance, especially if DO levels fall below 60% oxygen saturation.

Keywords: Sea bass, Blood smear, Gills, Brain, Erythrocytes measurements, Differential leukocyte count

1 Introduction

Dissolved oxygen (DO) is one of the most important limiting factors in intensive culture [1]. Adequate DO levels are very important for the welfare and development of fish [2-5]. DO is also the most important parameter of water treatment in aquaculture facilities and the supply requirements approach the mass rate of elimination addition to feed per day [6]. Complex seasonal, spatial and temporal variations in DO levels exist within sea cages in salmon farms [5,7]. In open sea fish cages, the major source of DO is from water exchange through sea currents as well as air diffusion through the air-water interface and turbulence caused by wind and waves [8]. In many cases, problems caused by reduced oxygen availability were reported at both marine and fresh water facilities [9-12]. Low concentrations of DO also indicated problems in Mediterranean aquacultures that produce sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) [13]. The relative oxygen consumption increases with temperature, activity, feed consumption and stress level [14]. In the Mediterranean region the increasing temperature is a limited factor for the oxygen solubility in water [14]. Aeration can be utilized both as an emergency tool and as a routine procedure, especially in critical periods, when related problems commonly occur. The type of aeration, dimensions of the devices and avoidance of total gas super-saturation are important factors; all of which must be considered. In the case of fish cages, the problem of low oxygen levels has generally been overlooked, and very few studies have been conducted. The general trend in the aquaculture industry is to believe that such problems only occur in poor locations for cages. Aeration in cages seems to be a solid alternative and an even better alternative to the addition of oxygen, as the

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transport of oxygen containers is not always technically feasible and can be economically difficult to justify [15]. Diffuser aerators use air produced by an air-compressor to inject air through diffusers located at the bottom of a pond or suspended in a water column. Understanding the spatial and temporal variability of key environmental variables within sea cages and how fish respond to them may enable modifications to sea cage environments and adjustments to management practices for improving production efficiency and fish welfare [5].

In fish cages, net pens reduce the strength of sea currents and, consequently, water exchange for farmed fish in the cages. For cultured red sea bream (*Pagrus major*), the required minimum dissolved oxygen is 5.7 mg/L, and oxygen levels below this amount may cause increased mortality [16]. Sea bass generally tolerate an O₂ deficit rather well. The physiological response (i.e., cortisol, glucose, and haematocrit) is similar in fish kept under hypoxic (3-4.5 mg O₂/L) and normoxic (7.4 mg/L) conditions [17]. Terova *et al.* [18] observed that acute hypoxia occurs at 1.9 mg/L after 4 hrs of exposure and that chronic hypoxia occurs at 4.3 mg/L after 15 days of exposure. According to Pichavant *et al.* [19], chronic hypoxia had no significant difference in haematocrit, erythrocyte number or mean corpuscular haemoglobin concentration in sea bass. Unfavourable oxygen conditions often occur with high water temperatures in late summer and autumn (> 25°C) in the Mediterranean Sea; these conditions normally cause reduced fish tolerance against low and fluctuating oxygen. Blood cells are directly related to the water oxygen concentration. Small erythrocytes enhance oxygen delivery. According to Lay and Baldwin [20] a functional basis for this relationship is found in larger surface area to volume ratios and shorter diffusion distances allowing more rapid oxygen transfer as erythrocyte volume decreases. Lymphocyte numbers can also be altered by hypoxia [21]. Thus, the fish erythrocyte size and number of leucocytes can be used as indicators for possible alterations to management practices for improving farmed water quality and hypoxic stress in fish.

Sea bass have four pairs of gill arches [22]. The gill arch is a curved osseous, or bony, structure from which double rows of paired primary lamellae or filaments radiate. Each of these primary lamella has a series of secondary lamellae branching off the primary lamellae in a perpendicular direction [23]. The efficient gas exchange of oxygen absorption and carbon dioxide release occurs across the epithelial surface of the secondary lamellae [23]. The efficiency of exchange, which, in the case of oxygen, is approximately 50-80%, is largely a function of the counter current exchange between blood and water

[22]. There could be gill morphological changes occurring after exposure to hypoxic conditions. According to Sollid *et al.* [24] these changes are a combination of reduced cell proliferation and an induction of apoptosis. The brain is about 2% of the total body mass and accounts for 20% of total oxygen consumption. 15-20% of blood flow travels from the heart to the brain. Due to its high metabolic demand, brain cells are extremely sensitive to oxygen deprivation [25].

This study investigates, in a farm-scale trial, how sea bass respond to a change in an environmental variable in a sea cage. The aim of this study is to determine the effect of the AirX aeration system on erythrocyte size and leucocyte differentiation of fish kept in sea cages, thus evaluating the AirX system performance in relation to the physiological response of farmed fish. A histological examination and morphometric measurements of the brain and gills were also conducted. These results can be utilized by the sea bass farming industry to improve production efficiency.

2 Materials and methods

2.1 Cages oxygenation

A system providing air (AirX diffuser system) was introduced in a 40 m circular and 8 m depth sea bass sea cage (aerated cage) in a small, owned by a family aquaculture farm located in Atalanti, Greece where the temperature can be critical in the summer period. An air diffusion based system (AirX) has been developed to control the dissolved oxygen levels in aquaculture sea cages (EU 7th Framework Programme, project number FP7-315412). The pressure of air at compressor output was kept at 4 bar. A circular hose of 24 m was placed at a depth of about 4 m. A second sea bass sea cage with the same dimensions but without the AirX system was used as a control cage. The stocking density in each cage was kept low at 8 kg/m³. The AirX diffuser system worked for a period of 37 days (from August 13th to September 18th, 2014) and provided air to the sea cage for 5 hours per day (10:00-12:00 and 21:00-24:00). Oxygen sensors were placed outside the cage and inside the cages at 2.5 m depth. Oxygen levels in both cages were measured every half hour by oxygen monitors. The oxygen levels in both cages were also monitored during July 10th to August 12th in order to detect any differences in oxygen levels before and after the operation of the AirX diffuser system. Fish growth rates and survival were also monitored. Sea bass weight measured on July 19th 2014 to obtain an estimation

of the biomass in the two cages. The average weights were 518 ± 32 g and 411 ± 35 g in the aerated and control cages, respectively.

2.2 Fish sampling

During the aeration period two fish samplings took place: 15 days after the AirX system was first used on the 28th of August (initial sampling) and at the end of the experimental trial, 18th of September (final sampling). Ten male adult sea bass specimen per sampling were taken, five from the aerated cage and five from the control cage. Fish were sampled with a dip net from the fish cages and immediately sacrificed by immersion on ice. Weight and total body length of each fish were measured. The total body length and body weight of the sea bass samples collected on August 28th from the control cage were 35.5 ± 1.8 cm and 512.8 ± 91.8 g, respectively, while the values for the sea bass from the aerated cage were 35.4 ± 1.0 cm and 466.5 ± 48.2 g, respectively. The total body weight and total body length measurements of the fish samples collected on September 18th were 511 ± 71 g and 38.8 ± 2.2 cm from the control and 638 ± 67 g and 36.4 ± 1.9 cm from the aerated cages, respectively.

2.3 Brain and gills histology

Whole brain and gill samples were taken from every fish, fixated into 10% formalin in filtered seawater for 24 h at 4°C and then immediately dehydrated in graded series of ethanol, immersed in xylol, embedded in paraffin wax. The samples embedded in paraffin wax were placed horizontally in biopsy type metal trays for histology. Gill and brain longitudinal sections of 5-7µm were mounted. After they had been deparaffinized, the sections were rehydrated, stained with hematoxylin and eosin, and mounted with Cristal/Mount. All tissues were examined under a microscope and any histological abnormalities were recorded. A digital camera adjusted to the microscope was used for acquiring histological section images. Primary lamella width, secondary lamellae length and the number of secondary lamellae per mm were measured.

2.4 Blood smears

Peripheral blood samples for every fish were taken from the caudal vessel with a syringe. Three blood smears

per fish were prepared. The blood smears were allowed to air dry, fixed for 2 minutes with 100% ethanol and stained with Giemsa. The slides were examined under oil-immersion at 100X magnification. For each fish differentiation and classification of the leucocytes took place. At least 100 leucocytes of each fish were recorded as one of three types: lymphocytes (L), granulocytes (G) and monocytes (M). In order to calculate the area of each erythrocyte, the major axis (Ma) and the minor axis (ma) of at least 124 erythrocytes of each fish were also measured. The area calculation was made with the formula:

$$A = \pi \frac{Ma}{2} \times \frac{ma}{2}$$

2.5 Statistical analysis

Dissolved oxygen level values and erythrocyte measurements were tested for normality using the Kolmogorov-Smirnov test. Statistical comparisons between the groups were made using the Mann-Whitney test (for non-normal distributions) and the T- test (for normal distributions).

3 Results

3.1 Oxygen conditions

No significant mortality was observed in either of the cages (more than 90% survival). There was no significant difference ($P > 0.05$) in the growth rate between the aerated and control cages (23.2% and 24.1%, respectively). The feed conversion efficiency (FCR) values were lower in the aerated cage, but were not significantly different ($P > 0.05$) (Makridis *et al.*, unpublished data). There were no statistically significant differences ($P > 0.05$) in the oxygen levels in the cages before introduction of the AirX diffuser system. No significant difference ($P > 0.05$) in the dissolved oxygen data measurements was observed at the start of the trial in both cages. During the working period of the AirX system, the oxygen level in the aerated cage significantly increased in comparison with the oxygen level in the control cage ($P > 0.05$) (Table 1). In the aerated cage, the lowest dissolved oxygen level measured was 67.5%, whereas it was 59.2% in the control cage in the last period (August – September). No hypoxic conditions could be assumed in any case. During the working period of the AirX system, there was little water turbulence and the fish showed no anxious behaviour. The absence of hypoxic conditions

and anxious behaviour led us to determine that there were only possible histological brain alterations, and we did not attempt to measure stress.

3.2 Blood parameters

The major axis, minor axis, and size area (μm^2) of erythrocytes as well as leucocyte differentiation were observed to follow the normal distribution (Tables 2 and 3, Fig. 1). The results are presented in Tables 2 and 3. All examined blood smears contained normal shaped erythrocytes and leucocytes. Only one fish from the first sampling of the aerated cage, appeared to have a very small number of erythrocytes (3-4 erythrocytes of a whole blood smear) with micronuclei (Fig. 2).

3.3 Gills and brain histology

The histological examination of sea bass gills revealed no morphological differences between the aerated fish and the control cage fish at either sampling, while the primary and secondary lamellae appeared normal. Chloride cells and mucous cells were present (Fig. 2). The gill morphometric measurements were also similar between the fish from the aerated cage and the control cage (Table 4). The examination of the parts of the brain (telencephalon, diencephalon, mesencephalon, and metencephalon) revealed normal histological images. Neuroglial cells and dendrites were observed. The granular layer, the ganglionic layer (with PURKINJE cells) and the molecular layer could also be observed in the cerebellum. The meninx appeared as a continuous

Table 1. Oxygen level (%) measurements. Results are given as median $\pm$ interquartile. n - number of the measurements during each time period.

	July, 10 – Aug, 12	Aug, 13 – Aug, 28	Aug, 29 – Sept, 18
Control cage O ₂ level [%]	97.05 ^a $\pm$ 10.53 n = 1586 min: 72.23 max: 117.5	84.09 ^b $\pm$ 12.67 n = 652 min: 59.16 max: 104.02	91.05 ^d $\pm$ 11.36 n = 1645 min: 59.16 max: 121.13
Aerated cage O ₂ level [%]	96.94 ^a $\pm$ 6.42 n = 1015 min: 78.27 max: 118.53	92.16 ^c $\pm$ 11.29 n = 652 min: 71.48 max: 120.69	91.91 ^e $\pm$ 8.73 n = 1645 min: 67.52 max: 120.69

Medians in a column followed by the same superscript are not significantly different ($P > 0.05$).

Table 2. Erythrocyte major, minor axis (μm) and area (μm^2) measurements. Results are given as mean $\pm$ SE, n = 124.

	Erythrocyte measurements	1 st sampling	2 nd sampling
Control cage	Major axis (μm)	11.86 $\pm$ 0.10	10.62 $\pm$ 0.09
	Minor axis (μm)	7.96 $\pm$ 0.06	7.26 $\pm$ 0.08
	Area (μm^2)	74.16 ^a $\pm$ 0.91	57.21 ^c $\pm$ 0.88
Aerated cage	Major axis (μm)	10.86 $\pm$ 0.10	10.20 $\pm$ 0.07
	Minor axis (μm)	7.43 $\pm$ 0.08	6.93 $\pm$ 0.06
	Area (μm^2)	63.42 ^b $\pm$ 0.96	55.59 ^c $\pm$ 0.67

Means in a column followed by the same superscript are not significantly different ($P > 0.05$).

Table 3. Differential leukocyte count (%) and classification. Results are given as mean $\pm$ SE.

		1 st sampling	2 nd sampling
Control cage	Lymphocytes	88 ^a $\pm$ 1.14	92 ^d $\pm$ 0.95
	Granulocytes	10.8 ^b $\pm$ 0.8	8 ^e $\pm$ 0.95
	Monocytes	1.2 ^c $\pm$ 0.49	0
Aerated cage	Lymphocytes	83 ^a $\pm$ 3.85	90.8 ^d $\pm$ 0.58
	Granulocytes	13.4 ^b $\pm$ 4.00	9.2 ^e $\pm$ 0.58
	Monocytes	1.6 ^c $\pm$ 0.24	0

Means of same types of leucocytes in a column followed by the same superscript are not significantly different ($P > 0.05$).

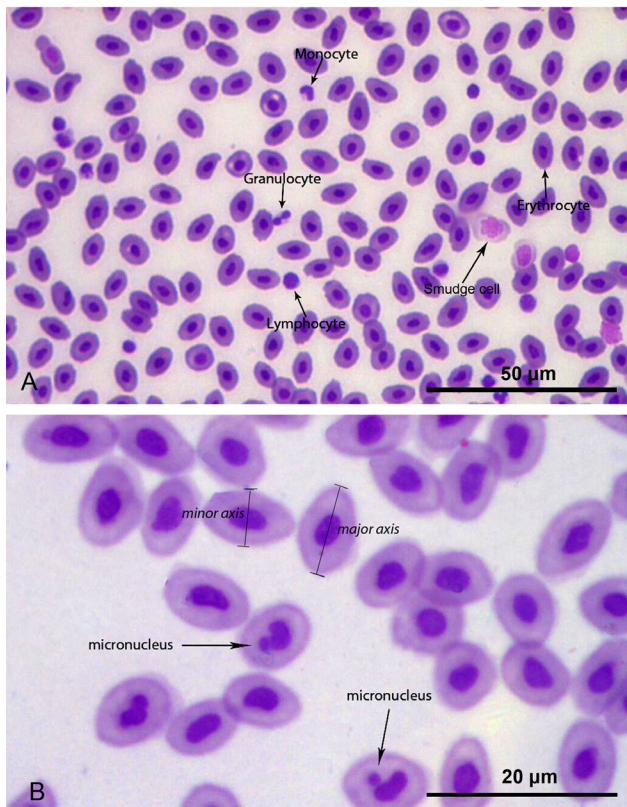


Figure 1. (A) Blood smear from the control sea bass cage with different cell types. (B) Blood smear from aerated sea bass cage with the presence of micronucleus.

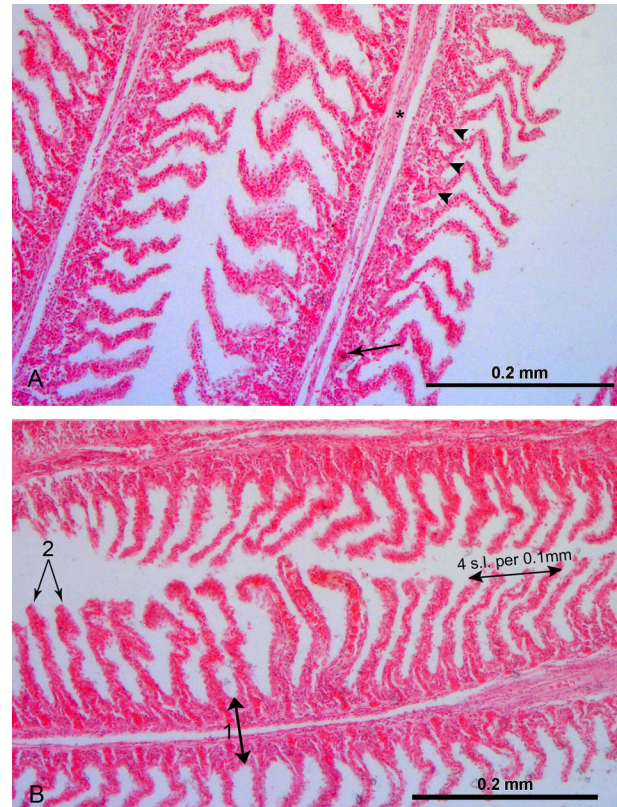


Figure 2. (A) Gill measurements from the control sea bass cage. Chloride cells (arrow), mucous cells (arrowhead) and oxygenated blood (star) are shown. (B) Gill measurements from the aerated sea bass cage. No histopathological alterations were detected. 1: primary lamella – 2: secondary lamellae (s.l.).

Table 4. Gill morphometric measurements (μm). Results are given as mean $\pm$ SE.

		1 st sampling	n	2 nd sampling	n
Control cage	Primary lamella width [μm]	254.98 ^a $\pm$ 7.80	109	235.29 ^a $\pm$ 10.63	107
	Secondary lamellae length [μm]	32.31 ^b $\pm$ 9.00	165	35.08 ^b $\pm$ 1.11	177
	Number of secondary lamellae per mm	32.20 ^c $\pm$ 0.61	50	32.72 ^c $\pm$ 0.35	57
Aerated cage	Primary lamella width [μm]	265.75 ^a $\pm$ 6.23	143	210.53 ^a $\pm$ 6.76	84
	Secondary lamellae length [μm]	31.48 ^b $\pm$ 1.19	165	36.55 ^b $\pm$ 0.93	149
	Number of secondary lamellae per mm	31.57 ^c $\pm$ 0.40	50	31.03 ^c $\pm$ 0.61	58

Means in a column followed by the same superscript are not significantly different ($P > 0.05$).

peripheral membrane without any rupture or distortion. No histological alterations or haemorrhagic signs were detected as a result of fish stress (Fig. 3).

4 Discussion

Since the early 1980s, the production of farmed gilthead sea bream (*Sparus aurata*) and sea bream (*Dicentrarchus labrax*) in Europe has increased, reaching approximately 265×10^3 tonnes in 2010 [26]. Approximately, 85% of the

production is from sea cages (Greece, Turkey and Spain), and the remainder is from land-based ponds (France, Italy, Portugal and Southern Spain) [27,28]. Metabolism in fish is influenced by numerous abiotic and biotic factors. For example, when feeding or oxygen availability is restricted, the best temperature at which fish grow is lower than when feeding and oxygen availability is not limited [29]. Dissolved oxygen is one of the most important limiting factors in intensive culture in both marine and fresh water species [1]. Pedersen [30] showed that growth rates of juvenile rainbow trout decreased if fixed levels of DO fell

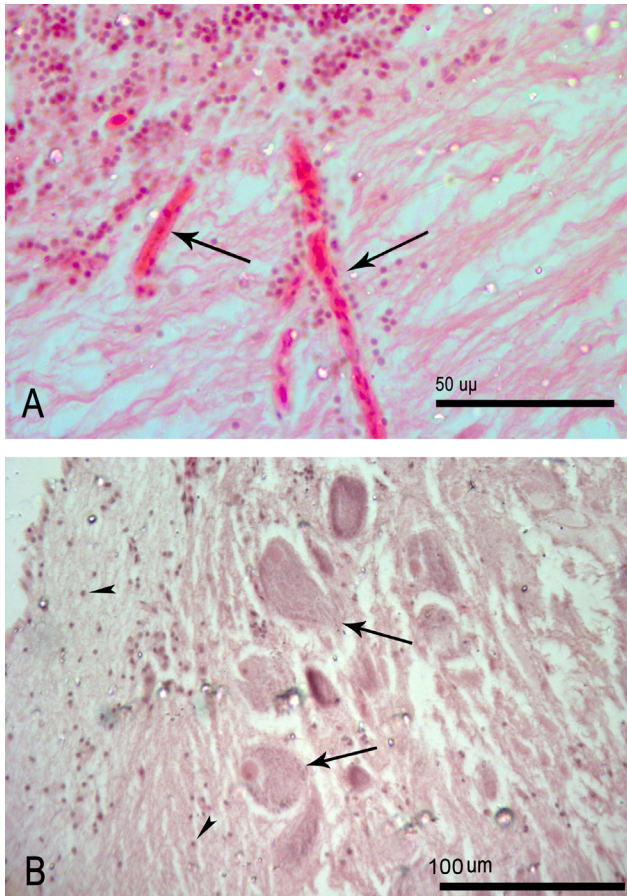


Figure 3. (A) Cerebellum section. Normal histological architecture with no haemorrhagic signs, as the capillaries (arrows) appeared intact. (B) Gray matter section. Neurons (arrows) and neuroglial cells (arrow head) appeared normal.

below 70% oxygen saturation and that trout ate less when fixed levels reached 60% oxygen saturation. Atlantic salmon held in seawater at 16°C and given fluctuating hypoxic saturation levels had reduced appetites, developed skin lesions, and had acute stress responses, reduced growth and mortalities [31]. The flow of the water currents in the area where the fish farm is established is very important, especially when decisions are made for the location of the fish farm. Hyperoxic conditions are unlikely to be found in the natural environment, whereas hypoxic conditions can and do occur particularly within fish farm cages [7]. Oxygen levels within the sea cages can be affected by the fish density in the cages and by any possible change in the currents that come through the cage [32]. Sub-optimal DO concentration deficit is a potential problem both in cages and ponds, and the risk of a harmful deficit is generally highest in late summer – early autumn at water temperatures above 25 – 27°C.

This study suggests that improved DO control by aeration, and thus stabilizing the concentration above

60% of saturation, would be a significant attempt to optimize the physiological performance of the fish. Although the survival and growth rates were not accelerated in the aerated cage, a lower FCR was calculated for sea bass kept in the aerated sea cage (Makridis *et al.*, unpublished data). One of the most important factors in aquaculture is to use feed as efficiently as possible to optimize returns. Thus, in practical terms for the farmer, the lower FCR values of the aerated cage translate into a lower amount of feed used in production and a quicker turnover of fish stocks compared to a non-aerated cage. Additionally, from a financial point of view, the farmer can quickly recover the capital and running cost of the aeration system. Similar results have been obtained in the aeration of cages with red sea bream in Japan [33]. The results from the samples obtained on August 28th showed that DO levels in the control cage were significantly lower than those in the aerated cage. Furthermore, the results on August 28th showed that the fish in the control cage had significantly bigger erythrocytes when compared with the fish in the aerated cage. According to Lay and Baldwin [20], this observation is due to the insufficient oxygen in the control cage, as small erythrocytes enhance oxygen delivery. Esteban *et al.* [34] stated that the erythrocyte maturation process in sea bass mainly involves a decrease in nuclear and cellular size, condensation of the chromatin and a progressive increase in the amount of haemoglobin. According to Lay and Baldwin [20], the haemoglobin concentration is negatively related to the erythrocyte volume and, therefore, small erythrocytes enhance oxygen delivery. They observed an inverse relationship between the metabolic rate and cell size, and spontaneous locomotor activity was frequently affected by reduced oxygen. In addition, teleosts with lower aerobic scopes possessed larger inefficient cells rather than reduced numbers of smaller efficient cells [20]. The smaller erythrocytes in fish in the aerated cage, due to the higher oxygen levels in the cage, may suggest that sea bass in this cage may have had a more active erythropoiesis, metabolism and swimming activity than the sea bass in the control cage. However, according to Nikinmaa [35], in hypoxic conditions there could be an increase in erythrocyte volume, which results from deoxygenation of haemoglobin and consequent decrease in its negative charge. But in normoxic conditions the larger erythrocytes in fish may suggest activation of transport pathways which mediate potassium and chloride efflux and restore cells volume [35]. As far as our DO levels showed no hypoxic conditions, further research is needed in order to determine the origin of the larger erythrocytes. Haematological parameters are important indicators of fish physiological

status, since these parameters are closely related to the response of the animal to the environment and contribute to assessment of fish condition [36]. Furthermore, a separate experiment in the laboratory in circular tanks, at a similar density (10kg.m^{-3}) was conducted by Araujo *et al.* [37] to evaluate the effect of different levels of dissolved oxygen (% of saturation) on sea bream performance. Their results showed a significant positive correlation between dissolved oxygen levels and SGR in sea breams. However, the mean value of haemoglobin, cortisol and glucose in the fish blood was not significantly different among the three levels of dissolved oxygen tested (40-60%, 60-80% and 80-100%) in commercial size sea bream and sea bass for 30 days. The AirX system uses the correct size of air microbubbles as an oxygen source, thus providing higher oxygen transfer efficiency. The results from the samples obtained on September 18th show that sea bass in the control cage did not have significantly larger erythrocytes when compared to sea bass in the aerated cage. This was because the sea bass in the control cage had a statistically significant increase in their water oxygen levels since August 28th (from 84% to 91%) due to an increase in the waves and winds during that period. Therefore, the erythrocytes of sea bass in the control cage had a smaller area size because the water in this cage was oxygen-enriched. According to Gravato and Santos [38], juvenile sea bass have approximately 0.1% normal micronucleus formation in their erythrocytes. An erythrocyte micronucleus is a consequence of no conjugated metabolites covalently bound to erythrocytic DNA. According to Gregory [39], the major axis, minor axis and area values are $9.5\text{ }\mu\text{m}$, $7.9\text{ }\mu\text{m}$ and $59.39\text{ }\mu\text{m}^2$, respectively, for sea bass erythrocytes whereas these values in diploid sea bass are $11.38\text{ }\mu\text{m}$, $8.34\text{ }\mu\text{m}$ and $71.31\text{ }\mu\text{m}^2$, respectively, according to Peruzzi *et al.* [40]. These results are similar to the results of this paper.

Fish live in intimate contact with their environment, and some environmental changes may be reflected in the components of their blood [41]. Gardner & Yerich [42] found seasonal variations in the morphology of erythrocytes, eosinophils and thrombocytes. Lymphocyte numbers can also be altered by hypoxia [21]. Danion *et al.* [43,44] examined blood from sea bass and found that lymphocytes comprised approximately 88% of the leucocytes, granulocytes comprised approximately 11% and monocytes comprised approximately 1%. The results by Danion *et al.* [43,44] were consistent with our results. Water oxygen levels, especially under hypoxic conditions, can alter the leucocyte number in fish blood. Studying the number of leucocytes in cultured fish, Enomato [21] found a decrease in lymphocyte numbers

during oxygen deficient conditions. Angelidis *et al.* [45] found similar results when rainbow trout were exposed to anoxic conditions. The total leucocyte number as well as lymphocyte number and percentage were reduced 24 h after the stressor was introduced. The percentage of lymphocytes, thrombocytes, neutrophils and haemoblasts of channel catfish were not significantly altered by prolonged sublethal hypoxia [46]. Our percent differentiation of the sea bass leucocytes in the aerated and control cages showed no significant difference, indicating the absence of hypoxic stress.

Most teleosts use gills as their main respiratory surface, although they also have accessory respiratory structures. Changes in environmental variables, such as the water oxygen content and temperature, can also cause fish to dramatically change the morphology of their gills in a rapid and reversible manner. Reports in the literature indicate that several species (*Oncorhynchus mykiss*, *Rutilus rutilus*, *Perca fluviatilis*, *Anguilla anguilla*, *Ambloplites rupestris*, *Micropterus salmoides*, and *Carassius carassius*) can reduce their respiratory surface area, to some extent, when challenged with unfavourable conditions (soft water, low pH or metals) and/or when exposed to low temperatures, which would reduce the oxygen demand [24,47-51]. The goldfish is extremely hypoxia-tolerant and has haemoglobin with a very high oxygen affinity [52]. Fish exposed to hypoxia during development may end up with a significant, but relatively modest, increase in respiratory surface area. An 18% increase in an African cichlid was recorded by Chapman [53].

Stress is an important factor for the welfare of fish. We maintained the air pressure in the water at 4 bar in order to have the least amount of water turbulence and to decrease the chance of exposing the fish to this stress factor. In mammals, stress resulted in a transient increase in anxiety, with no detectable brain tissue damage [54]. Hypoxia can lead to brain damage. Cell changes, consisting of microvacuolation, appear to be fully reversible in the early post-hypoxic period. Irreversible cell death, coagulative cell change and edematous cell change can be developed in a later phase [55]. High concentrations of oxygen for a period of hours during a specific period of development caused an apoptotic neurodegenerative reaction that deleted large numbers of neurons in the developing brain [56]. Unfortunately, there is a gap in the histological alterations in the fish brain after stress, hypoxia or hyperoxia. The teleost brain is quite similar to the brains of higher animals and is usually divided into five divisions: the telencephalon, the diencephalon, the mesencephalon, the metencephalon (cerebellum) and the medulla oblongata [23]. Further research is needed,

especially in the case of sea cages, where the difficulty of reduced oxygen levels has been underestimated. Aeration in cages seems to be a solid alternative and better than addition of oxygen, as the transport of oxygen containers is not always feasible and can be economically difficult to justify. Improved DO control by aeration and stabilizing the concentration above 60% of saturation would be a significant attempt to optimize the conditions of the fish.

5 Conclusions

During the working period of the AirX system, the oxygen level in the aerated cage was significantly increased in comparison to the oxygen level in the control cage. Mortality, growth rates and leucocyte differentiation were not affected by aeration. Fish gills and brain histology appeared normal after the short time of aeration. However, aeration had an impact on the physiological parameters, such as erythrocyte size. The lower amount of oxygen in the sea cage led to larger erythrocytes. DO controlled and stabilized by aeration to a concentration above 60% saturation, optimizes the conditions for the fish. This study demonstrated that an air diffuser system could improve the water quality in sea cages and could enhance sea bass physiological performance, especially when DO levels fall below 60% oxygen saturation. Aeration, diffusion-based aeration and mapping of DO deficit problems in cage farms are considered to be very important factors for commercial fish farms.

Authors' contributions: PB designed the study, carried out part of the sampling, the histological and data analysis and the writing of the paper. EM designed the study, carried out part of the sampling, the histological and data analysis and contributed in the writing of the paper. EN carried out the oxygen measurements, the sampling and part of the histology and data analysis. PM contributed to the design of the study and the AirX diffuser system and participated in the oxygen measurements, the sampling and the writing of the paper. HG contributed to the design of the study, the AirX diffuser system and the oxygen measurements and the sampling. AB contributed to the design of the study, the AirX diffuser system and the oxygen measurements. MG contributed to the design of the study and provided the AirX diffuser system.

All authors read and approved the final manuscript.

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Ethical standards: This study was reviewed and approved by the University of Thessaly for ethics. International guidelines for animal welfare were followed.

Competing interests: The authors declare that they have no competing interests.

Conflict of interest: The authors declare nothing to disclose.

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