Haematological and immunological responses of sea bass (*Dicentrarchus labrax*) to a short-term exposure to increased water levels of nitrate

Vectesi D.[1]; Kotzamanis I. [1]; Henry M. A[1].; Vatsos I. N. [2*]

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1- Laboratory of Fish Nutrition and Pathology, Institute of Aquaculture, Hellenic Centre for Marine Research, Athens 16610, Greece.

2- Ichthyology Laboratory, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, PO Box 395, 54124, Greece.

* Corresponding author's email: ivatsos@vet.auth.gr

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Fish reared under intensive culture conditions very often face stressful adverse conditions which either do not exist in nature, for example living in extremely high stocking densities, or they are quite unlikely, for example increased water ammonia levels (Huntingford et al. 2006).

Nitrate (NO_3^-) which is the ionized form of nitric acid (Cheng and Chen, 2002) and salts, like sodium nitrate, are readily soluble in water and completely dissociated. It is produced by a two-step process called 'nitrification' (Hargreaves and Tucker, 2004). During this process, ammonia, which is either excreted from fish or produced by the decomposition of the organic matter in the water, is first oxidized to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-). The nitrification rate can be affected by many factors such as water temperature and available diluted oxygen (Hargreaves and Tucker, 2004).

In most aquaculture systems, nitrate levels are below 50 ppm, but in intensive culture systems, especially in recirculation systems, nitrate levels often exceed 100 and sometimes approach 500 ppm causing problems for fish, Nitrate, compared to nitrite, is generally considered to be of low toxicity to fish, due to its substantially lower brachial permeability (Camargo et al. 2005). However, on many occasions and especially in younger fish, which are generally more sensitive to toxicants, nitrate can cause increased mortalities (Camargo et al. 2005). The tolerance to nitrate depends on the fish species as well as the period of exposure. Thus, a 30 day exposure of chinook salmon (*Oncorhynchus tschawytscha*) fry to 20 ppm resulted in significantly increased mortality, while increased mortality in rainbow trout (*Oncorhynchus mykiss*) fry was observed when the concentration of nitrate in the water exceeded 10 ppm (Kincheloe et al. 1979). For coho salmon (*Oncorhynchus kisutch*), exposure to higher nitrate concentration (30 ppm) caused increased fry mortalities (Kincheloe et al. 1979). On the other hand,

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however, short-term exposure of fish to nitrate seems to induce less significant effects. For example, larvae of four marine fish species (*Gaidropsarus capensis*, *Heteromycteris capensis*, *Diplodus sargus* and *Lithognathus mormyrus*) were found to be tolerant to a 24h exposure to very high nitrate levels with LC_{50}, ranging from 4200 to 8400 ppm (Brownell, 1980). Similar findings have been reported by Camargo et al. (2005) for seventeen fish species, marine and freshwater. The exact mechanism by which the increased levels of nitrate induce increased mortalities is still not clear (Hamlin, 2006). As Browell (1980) suggested, the increased mortalities observed in fish exposed to extremely high concentrations of nitrate can be attributed to significantly increased osmolarity of the final solutions (over 40 ‰), but this is not the case when low concentrations of nitrate are examined. Camargo et al. (2005) reviewing previous studies on the toxicity of nitrates, reported that when nitrate enters the body of fish it interferes with the ability of the blood pigments to carry oxygen.

Sea bass (*Dicentrarchus labrax*) is a commercially important euryhaline marine fish species which is intensively cultured in the Mediterranean area. The first stages of its culture take place in on-shore tanks and the last stages in sea cages. Lately, the potential of rearing sea bass only in tanks, in extremely high stocking densities, has been investigated (Sammouth et al. 2009). As it was mentioned above, under these conditions increased water levels of nitrate can occur and even if no mortalities are observed, the resulting stress can compromise the fish's immune system making them susceptible to many opportunistic bacteria present in the water. A previous study carried out by Vatsos et al. (2010) showed that short-term exposure of sea bass of the same size to 100 and 700 ppm induced stress to fish as indicated by the increased number of skin mucous cells in the exposed fish. The number and the size of these cells are considered a reliable stress index. In that study however, no assessment of the effects of nitrate on any hematological or immunological was attempted.

In the present study, the effects of a 48 h exposure of sea bass to 100 and 700 ppm of nitrate on some immunological as well as haematological parameters were assessed. These two concentrations were selected on the basis that they can be observed under intensive farming conditions (Camargo et al. 2005). For this purpose, 45 sea bass (mean weight 155.6 ± 10.3 g SD) were purchased from a commercial fish farm, located in the western Greece. It should be noted that although fry and fingerlings tend to be more susceptible to increased levels of nitrate, in the present study bigger fish were used so that adequate blood sample from each fish was collected and thus individual values were used for the statistical analysis. The fish were randomly divided into 3 groups and placed into nine 100 L tanks (3 replicates in each group; 5 fish per tank) filled with sea water. The fish remained in these tanks for one week to acclimatize and the water parameters during this period were: water temperature 23.7 to 24.5 °C, salinity 38‰ and pH 8.01. An artificial regime of 14 h light and 10 h darkness was applied. The water in each

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tank was constantly aerated using air stones to over 85% oxygen saturation. The fish were fed twice a day ad libitum.

Just prior to the exposure, the water flow in each tank was stopped and nitrate in the form of sodium nitrate was added to the water. In the first three tanks (Group A) the concentration of nitrate in the seawater was adjusted to 100 ppm, while in the other 3 tanks (Group B) the concentration was adjusted to 700 ppm. The fish in the remaining 3 tanks (Group C) were kept as controls (nitrate tank concentration 1.1 ± 0.3 ppm SD). The final concentration of the nitrate in all the tanks was confirmed using a HACH, DR/890 portable colorimeter. During the experimental period (48 hours) the fish were not fed and the water in each tank was constantly aerated. All the water parameters were similar to those recorded prior to exposure. The overall behavior of the fish was observed daily and the mortalities were recorded.

At the end of the experimental period all fish were anaesthetized using phenoxyethanol (0.25 ppm) and immediately 2 ml blood samples were collected from the caudal vein. For each blood sample, half of the blood sample was left at 4 ^{o}C to coagulate in order to be used in the determination of the bactericidal and lysozyme activities of serum, while the other half of the sample was heparinised (150u/ml) to be used in the haematological assessments and for the determination of the whole blood chemiluminescence activity.

All procedures carried out in the present study followed the international guidelines for animal welfare and treatment.

The haematological parameters that were examined were measured as described by Rigos et al. (2010). Red blood cells count (RBC) and white blood cell count (WBC) were performed using Dacie's fluid; haemoglobin concentration (Hb) was obtained using a Drabkin's reagent and haematocrit (Hct) was obtained by centrifugation of capillary tubes.

The following parameters were deduced from the measured haematological parameters using the equations below:

- Mean corpuscular haemoglobin (MCH, pg) = Hb (g/100 ml) x 10 / RBC (x 10^6/mm^3)

- Mean corpuscular volume (MCV, fl) = Hct (%) x 10 / RBC (x 10^6/mm^3)

- Mean corpuscular hemoglobin concentration (MCHC, g/100ml) = Hb (g/100 ml) x 100 / Hct (%).

The immunological parameters that were examined in the present study included whole blood chemiluminescence assay, the serum bactericidal activity and the serum lysozyme activity.

Whole blood chemiluminescence assay. For this assessment, the method of Marnila et al. (1995) was used with a slight modification. In brief, 5.2 µl of heparinised blood was added to 1 ml of gHBSS (complete HBSS enriched with 0.1% gelatine, 5 units/ml of heparin and 100 IU ml^{-1} of Penicillin/Streptomycin) and 40 µl of 5 mM luminol in 1M potassium hydroxide and 1M boric acid (pH 9.0). 275 µl of this mixture was added in triplicate wells of a white 96-wells flat-

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bottomed microplate (Nunc). After stabilization of the background chemiluminescence for 10 minutes at room temperature, 25 µl of Zymosan at 5 mg/ml in complete HBSS was added in each well to trigger the chemiluminescence response. Chemiluminescence was read every 5.5 minutes for 120 minutes at 27 °C by GeniosPro luminometer (Tecan, Austria). Results were expressed as the peak CL in relative luminescence units (rlu).

Serum bactericidal activity. The method used was adapted from Nikoskelainen et al. (2002). Blood was left to coagulate overnight at 4°C and centrifuged twice at 9000 g for 10 min. Fish sera were diluted with Phosphate Buffer Saline (PBS) and 50 mM $Mg^{2+}Ca^{2+}$ (pH 7.4) and increasing amounts (0-100 µl) of the diluted serum were added to 12 wells of a white 96-wells flat-bottomed microplate (Nunc) giving final serum concentrations ranging from 0 to 40 µl/ml for each fish. The volume in each well was adjusted to 100 µl with PBS and $Mg^{2+}Ca^{2+}$. The bacteria used (*E.coli* K12pEGFLPLucTet kindly provided by S. Verho, University of Turku, Finland) were grown overnight to log-phase at 37 °C in LB broth containing 10 µg tetracycline ml^{-1} and their concentration was adjusted to give an OD_{450nm} of 0.1. Fifty µl of this bacterial suspension was added to each well. A 3 h- incubation at 23°C allowed bacterial killing. D-luciferin (Synchem, Germany) was added to each well (100 µl at 0.5 mM in 0.1 M citrate buffer, pH 5.0) and the emitted luminescence (rlu) measured by GeniosPro luminometer (Tecan, Austria) was proportional to the number of live bacteria. The maximal bactericidal capacity (MBC) of the serum was the lowest concentration of serum which killed the bacteria. Below this serum concentration, no further bacterial killing occurred.

The maximal bactericidal capacity was thus calculated as follows:

- MBC = 100-(rlu obtained with serum at the final concentration x 100) / rlu without serum.

The concentration of serum giving 50% of bacterial killing (IC_{50}) was transformed in units/ml using the following equation: Bactericidal activity (units/ml) = $1000/IC_{50}$.

Serum lysozyme activity. The method used in this study to estimate the lysozyme activity was adapted from Cuesta et al. (2002). 25 µl of fish serum was added to triplicate wells of a transparent 96-flat bottomed well microplate (Greiner). 200 µl of *Micrococcus luteus* at 0.75 mg/ml in 0.1M sodium phosphate/citric acid buffer (pH 5.8) was added to each well. The kinetic of decrease of OD was measured at 450 nm every 3 min for 20 min. Lysozyme activity (units/ml serum) was deduced from the slope considering that 1 unit corresponded to a decrease of OD of 0.001/min.

For all parameters tested, the individual values of the three replicates in each group were compared and found to be similar using chi square test. Thus it was decided to pool all replicates in each group, in order to gain statistical power. All data from the individual measurements were then tested for normality and homogeneity of variance prior to be subjected to one–way ANOVA using Kolmogorov-Smirnov

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and Levene tests, respectively. Significant differences between means were determined using Tukey's test. The level of significance was set at $P < 0.05$.

All statistical tests were performed using the General Linear Model (STATISTICA version 7.0).

The values of the chemiluminescence activity were ln-transformed in order to homogenise the variances. No transformation tested could normalise the variances of the results for the lysozyme activity and MBC of sera. The non-parametric Kruskal-Wallis test was then performed.

No mortality was observed in any group and no statistical difference was noted between the three groups of fish in all the parameters examined. Concerning the haematological parameters, the concentration of haemoglobin tended to increase in the fish exposed to nitrate (Table 1). In addition, in the same groups of fish, the total number of white blood cells tended to decrease, while the number of the red blood cells slightly increased. Slight alterations were recorded in the MCV, MCH and MCHC (Table 1).

Table 1: Effect of the two levels of nitrate on the haematological parameters. Values represent means of 15 fish per group + standard error (SD)

Factor	Control	100 ppm	700 ppm
RBC *10^6	3.45±0.51	3.66±0.60	3.62±0,50
WBC *10^4	12.97±1.64	10.48±4.16	10.64±2.71
Lymphocytes%	75.56±11.2	73.38±7.53	68.92±17.57
Thrombocytes%	12.40±9.65	17.88±9.26	16.92±12.7
Granulocytes%	10.36±8.26	7.42±3.36	12.05±5.54
Monocytes%	1.66±1.21	1.28±0.70	2.10±1
Hct %	32.66±4.56	29.81±4.81	32.83±3.83
Hb (mg/ml blood)	116.27±27.7	124.16±15.5	120.17±10.7
MCV (fl/cell)	94.60	79.32	88.87
MCH (pg/cell)	36.45	32.76	34.54
MCHC (g/100ml)	38.53	41.30	38.87

Thus a decrease in the MCV and MCH accompanied by an increase in the MCHC indicated that the red blood of the fish exposed to nitrate tended to shrink. The alteration appeared to be more pronounced in the fish exposed to 100 ppm nitrate. Concerning the immunological parameters, the whole blood respiratory burst activity measured by chemiluminescence tended to be stronger in the fish maintained in both concentrations of nitrate, but the bactericidal activity of the serum and the maximal killing capacity slightly decreased (Figure 1). Regarding the serum lysozyme activity, no statistical difference and no clear trend were observed.

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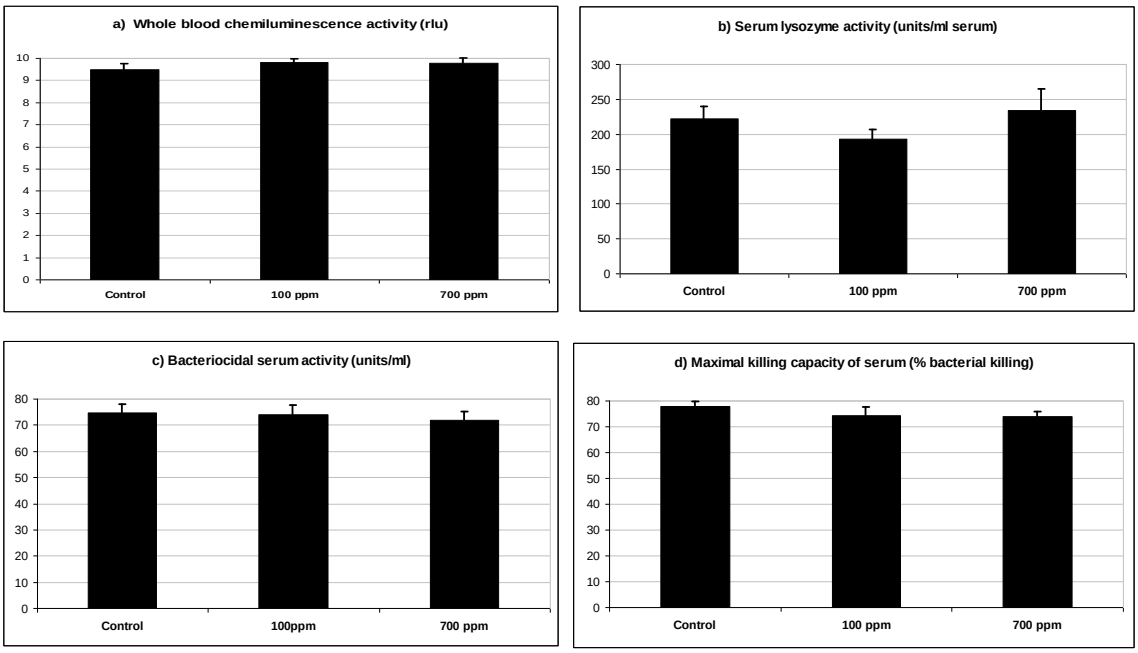


Figure 1: Effect of the two levels of nitrate on the immunological parameters. Values represent means of 15 fish per group + standard error (SE)

It is known that the nitrate can enter the bodies of fish and shellfish, possibly through a brachial uptake system and then accumulate in many tissues. For example in shrimp, nitrate can be accumulated in the haemolymph, gills muscles and hepatopancreas (Cheng and Chen, 2002). In fish, nitrate can affect the gills, the liver, the intestine and the kidney, and at high concentrations can cause inflammation and necrosis in these organs (Shimura et al. 2004). These alterations are similar to those observed in ammonia poisoning. Under normal conditions, nitrate is generally considered of low toxicity to fish. However, many studies have indicated that short-term exposure of fingerlings and older fish of many fish species to high concentrations of nitrate, usually over 500 ppm, can cause significant mortalities (Camargo et al. 2005). On the other hand, short-term exposure of fry to 1-200 ppm (depending on the species) can also provoke significant mortalities (Camargo et al. 2005). However there is limited information on the subclinical effects of nitrate on fish and until now, no safe nitrate levels have been established for many aquatic animals, although an ambient nitrate level of 20 ppm is generally considered safe for most marine fish species, while 2 ppm is considered appropriate for most freshwater species (Camargo et al. 2005). Recently, reviewing previous studies, Hamlin et al. (2008) reported that sublethal concentrations of nitrate can affect the endocrine system, the metabolism and the reproduction in various fish species. In the present study, although no mortalities were recorded, the fish exposed to increased levels of nitrate exhibited notable alterations (though not statistically significant) in some of the parameters examined.

The assessment of many haematological parameters can provide important information on the overall health

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status of fish and particularly their physiological response to many adverse and stressful environmental conditions (Danabas et al. 2010). Among the haematological parameters examined in the present study, the decrease in the circulating WBC, the increase in the hemoglobin (Hb) concentration and the increase in the percentage of thrombocytes in the fish exposed to nitrate is of particular significance. These results are in agreement with those of other studies on acute stress, as for example the one conducted by Benfey and Biron (2000). In that study, the authors studying the effects of acute stress in rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*), after exposing the fish to handling and confinement, two quite common stressful situations that occur under culture conditions, also noted a decrease in WBC and an increase in Hb concentration. Especially for the decrease in WBC, the authors, based on previous studies, concluded that the nature of the stressor and the fish species involved affect the level of the decrease. Maule and Schreck (1990) found that the lymphopenia, which is commonly observed in many cases of acute stress in fish, is probably the result of a redistribution of these cells, which tend to accumulate in certain organs, such as the thymus and the kidney. In our study, the number of lymphocytes clearly tended to decrease as the concentration of the ambient nitrate increased, indicating a serious immune deficiency. On the other hand, in cases of chronic stress, a depletion of lymphocytes from all lymphoidal tissues is usually observed (Benfey and Biron, 2000).

In the present study, the number of red blood cells increased slightly in the fish exposed to nitrate. This increase was accompanied by a decrease in their size, as the assessment of MCV, MCH and MCHC indicated. This finding (increased number of red blood cells of smaller volume) is a non-specific response, observed commonly in fish exposed to various stressors and is usually the result of the spleen's contraction, which releases new red blood cells of smaller MCV into the circulation (Kori-Siakpere and Ubogu, 2008).

The increase in the Hb concentration that was observed in both groups of fish exposed to nitrate could be related with the increase in the number of red blood cells. However, since this increase was not accompanied by a significant increase in Hct it appears that haemolysis may have also occurred. It is known that in many cases of exposure to various toxicants, increased water uptake by the gills is observed resulting in haemodilution and destruction of the red blood cells (Wendelaar Bonga and Lock 1992).

The increase in the percentage of thrombocytes that was observed in this study is also a non-specific response of fish to acute stress. Frisch and Anderson (2000) suggested that this is a catecholamine-related response and serves fish to prepare them for any potential blood loss.

It is well established that stress can cause immunosuppression in fish, which in turn can increase their susceptibility to many diseases (Ellis and Manning, 1989; Pickering, 1992; Mellergaard, and Nielsen,

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1995). However, depending on the nature of the stressor (e.g. environmental, physical, or chemical) and the period of exposure, the effects of stress on the fish immune system can vary. For example, Ortuno et al. (2002) found no effect of physical disturbance on the serum complement activity nor on the head-kidney leucocytes respiratory burst in sea bream (*Sparus aurata*). On the other hand, the authors noted that crowding and anaesthesia depressed the complement activity and prolonged exposure to air caused a significant reduction in the respiratory burst activity one day after the exposure. Similarly, in catfish, short-term exposure to sub lethal hypoxia decreased complement activity (Welker et al. 2007). In the present study, a slight decrease in the ability of the serum of the exposed fish to kill bacteria was observed, as assessed by the bactericidal activity and the maximal killing capacity of the serum. This finding however, indicates that exposure of fish to similar conditions can potentially affect the course of any infectious disease, especially when other stressful conditions co-exist.

In conclusion, in the present study a 48 h exposure of sea bass to 100 and 700 ppm nitrate did not evoke statistically significant changes in the immunological and haematological parameters that were examined. Nonetheless, the alterations which were noted indicated that the fish exhibited the general non-specific physiological responses observed in any acute stressful condition. It is believed that the period of exposure and / or the concentration used were not sufficient enough to elicit notable responses. However, under the intensive farming conditions that commonly exist in land-based facilities, such concentrations can be found and thus the results of this study may provide a realistic picture of the physiological response of the fish. It should also be noted that under intensive culture conditions the increased concentrations of nitrate in the water are usually accompanied by other conditions, as for example increased stocking density, or reduced water renewal, which can induce additional stress to fish. Thus, under these conditions the combined effects of these stressors on the physiology of fish and particularly on the immune system may become significant.

References

Benfey, T. J. and Biron, M., 2000. Acute stress response in triploid rainbow trout (*Oncorhynchus mykiss)* and brook trout (*Salvelinus fontinalis*). *Aquaculture*, 184, 167–176.

Blaxhall, P. C. and Daisley, R. W., 1973. Routine haematological methods for uses with fish blood. *Journal of Fish Biology*, 5, 771-781.

Brownell, C. L., 1980. Water quality requirements for first-feeding in marine fish larvae. I. Ammonia, nitrite and nitrate. *Journal of Experimental Marine Biology and Ecology*, 44, 269-83.

Camargo, J. A., Alonso, A. and Salamanca A., 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere*, 58, 1255-1267.

Cheng, S. Y. and Chen, J. C., 2002. Accumulation of nitrate in the tissues

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[DOR: 20.1001.1.15622916.2012.11.2.18.6]

of *Penaeus monodan* following elevated ambient nitrate exposure to combined elevated nitrite and nitrate. *Aquatic Toxicology*, 61, 181-193.

Cuesta, A., Ortuno, J., Rodriguez, A. Esteban, M. A. and Meseguer, J., 2002. Changes in some innate defence parameters of sea bream (*Sparus aurata* L.) induced by retinol acetate. *Fish Shellfish Immunology*, 13, 279-291.

Danabas, D., Yildirim, N. C., Gulec, A. K., Yildirim, N. and Kaplan, O., 2010. An investigation on some haematological and biochemical parameters in *Capoeta trutta* (Heckel 1843) from Munzur River (Tunceli, Turkey). *Journal of Animal and Veterinary Advances*, 9(**10**), 2578-2582.

Ellis, A. E. and Manning, M. J. 1989. Environmental factors, immunization and diseases control. *Developmental and Comparative Immunology*, 13, 395-396.

Frisch, A. J. and Anderson, T. A., 2000. The response of coral trout (*Plectropomus leopardus*) to capture, handling and transport and shallow water stress. *Fish Physiology and Biochemistry*, 23, 23–34.

Hargreaves, J. A., Tucker, C. S., 2004. Managing ammonia in fish ponds. SRAC Publication, No. 4603.

Hamlin, H. J., 2006. Nitrate toxicity in Siberian sturgeon (*Acipenser baeri*). *Aquaculture*, 253, 688-693.

Hamlin, H. J., Moore, B. C., Edwards, T. M., Larkin, I. L. V., Boggs, A., High, W. J., Main, K. L. Et al., 2008. Nitrate-induced elevations in circulating sex steroid concentrations in female Siberian sturgeon (*Acipenser baeri*) in commercial aquaculture. *Aquaculture*, 281, 118-125.

Huntingford F. A., Adams C., Braithwaite V. A., Kadri S., Pottinger T. G., Sandøe P. and Turnbull J. F., 2006. Current issues in fish welfare. *Journal of Fish Biology*, 60, 332-372.

Kincheloe, J. W., Weoemever, G., Koch, O. L., 1979. Tolerance of developing salmonid eggs and fry to nitrate exposure. *Bulletin Environmental Contamination and Toxicology*, 23, 575-8.

Kori-Siakpere, O., and Ubogu, E. O., 2008. Sublethal haematological effects of zinc on the freshwater fish, *Heteroclarias* sp. (Osteichthyes: Clariidae). *African Journal of Biotechnology*, 7 (**12**), 2068-2073.

Marnila, P., Tiiska, A., Lagerspetz, K. and Lilius, E. M. 1995. Phagocyte activity in the frog *Rana temporaria*: whole blood chemiluminescence method and the effects of temperature and thermal acclimation. *Comparative Biochemistry and Physiology*, 111A, 609-614.

Maule, A. G. and Schreck, C. B., 1990. Changes in numbers of leukocytes in immune organs of juvenile coho salmon after acute stress or cortisol treatment. *Journal of Aquatic Animal Health*, 2, 298–304.

Mellergaard, S. and Nielsen, E., 1995. Impact of oxygen deficiency on the disease status of common dab, *Limanda limanda*. *Diseases of Aquatic Organisms*, 22, 101-114.

Nikoskelainen, S., Lehtinen, J. and

[Downloaded from jifro.ir on 2026-11-12]

[DOR: 20.1001.1.15622916.2012.11.2.18.6]

Lilius, E. M., 2002. Bacteriolytic activity of rainbow trout (*Oncorhynchus mykiss*) complement. *Developmental and Comparative Immunology*, 26, 797-804.

Ortuno, J., Esteban, M. A. and Meseguer, J., 2002. Lack of effect of combining different stressors on innate immune responses of sea bream (*Sparus aurata*). *Veterinary Immunology and Immunopathology*, 84, 17-27.

Pickering, A. D., 1992. Rainbow trout husbandry: management of the stress response. *Aquaculture*, 100, 125-139.

Rigos, A., Samartzis, M., Henry, E., Fountoulaki, E., Cotou, J., Sweetman, S., Davies, I. and Nengas, I., 2010. Effects of additive iron on growth, tissue distribution, haematology and immunology of gilthead sea bream, *Sparus aurata*. G. *Aquaculture international*, 18(**6**), 1093-1104.

Sammouth, S., d'Orbcastel, E. R., Gasset, E., Lemarié, G., Breuil, G., Marino, G., Coeurdacier, J. L. et al., 2009. The effect of density on sea bass (*Dicentrarchus labrax*) performance in a tank-based recirculating system. *Aquaculture Engineering*, 40(**2**), 72-78.

Shimura, R. Ma, Y. X., Ijiri, K., Nagaoka, S. and Uchiyama, M., 2004. Nitrate toxicity on visceral organs of medaka fish, *Oryzias latipes* : aiming to raise fish from egg to egg in Space. *Biological Sciences in Space*, 1, 7-12.

Vatsos, I.N., Kotzamanis, Y., Henry, M., Angelidis, P. and Alexis, M.N., 2010. Monitoring stress in fish by applying image analysis to their skin mucous cells. *European Journal of Histochemistry*, 54(**2**), 107-111.

Wendelaar Bonga, S. E. and Lock R. A. C., 1992. Toxicants and osmoregulation in fish. *Netherlands Journal of Zoology*, 42, 478-493.

Welker, T. L., Lim, C., Yildirim-Aksoy, M., Shelby, R. and Klesius, P. H., 2007. Immune response and resistance to stress and *Edwardsiella ictulari* challenge in channel catfish, *Ictalurus punctatus*, fed diets containing commercial whole-cell or yeast subcomponents. *Journal of World Aquaculture Society*, 38, 24-35.

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