The influence of acute handling stress on some blood parameters in cultured sea bream (*Sparus aurata* Linnaeus, 1758)

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Abstract

The effect of acute handling stress on haematological profile, blood glucose and lactate (secondary stress markers) in cultured sea bream *Sparus aurata* was evaluated. Sixty six *Sparus aurata* were used and equally divided into two groups (A and B). Group A was not subjected to stress, Group B was subjected to acute handling stress. From each fish, biometric data and blood samples were collected to evaluate haematological profile, blood glucose and lactate. Unpaired t-test Student was applied to evaluate possible differences in parameters between the two groups. Red blood cells, haematocrit, haemoglobin, white blood cells (WBC), glucose and lactate showed an increase ($P<0.05$) in Group B compared with Group A, while mean corpuscular volume decreased ($P<0.05$) in Group B. The results highlight the role of studied parameters in monitoring the stressful conditions of aquaculture production which affect animal welfare and fish products quality.

Introduction

Acute or chronic stressors, unavoidable consequences of normal hatchery practices in aquaculture, strongly affect fish physiology including growth, reproduction and welfare (Eslamloo *et al*., 2014). Management practice has been recognised as a key to profitable and sustainable fish farming. This has led to the application of manipulation strategies to maximise fish production in the culture environment. Some aquaculture procedures commonly used, such as acclimation and handling, affect the stress response of fish (Ashley, 2007). Fish's responses to stress are known to cause alteration of blood characteristics, reduction of growth rate and reproductive process, suppression of immune system, disruptions in metabolic activities and alteration of fish products quality (Wells and Pankhurst, 1999). The primary stress response in fish involves the release of catecholamines and the activation of the hypothalamic-pituitary-interenal. Hypothalamic-pituitary-interenal activation results in energy source mobilization, depletion of glycogen stores, and an increase in plasma levels of glucose, along with high muscle activity, anaerobic glycolysis and an increase in plasma lactate. Therefore, the levels both of glucose and lactate in plasma are often used to assess stress levels (Arends *et al*., 1999; Acerete *et al*., 2004).

Haematological parameters are important to assess the physiological status of fish and to monitor stress and pathological changes (Fazio *et al*., 2012b). A basic knowledge of the haematology represents a valuable guide to assess the condition of aquatic organisms and it is widely used as indicator of environmental stress. Haematological and haematochemical responses to a particular stress factor are quantitatively related to the severity and longevity of the stress.

The aim of the present study was to evaluate the effect of acute handling stress on haematological profile, blood glucose and lactate (secondary stress markers) in cultured sea bream *Sparus aurata*.

Materials and Methods

A total of 66 adult sea bream (*Sparus aurata*) in excellent health status, taken from an onshore aquaculture system located on the South-Eastern coast of Sicily, was used in this study. Water physico-chemical parameters measured in the tank using a multiparametric probe C203 (Hanna Instruments, Woonsocket, RI, USA) were the following: temperature=21°C, salinity=38‰, dissolved oxygen=5 ppm.

The following experimental protocol was used: fish were divided into two groups (Groups A and B), of 33 fish each. During the experimental protocol the two groups were transferred in two tanks, respectively, equipped with aerators. The tanks were in a flow-through system. Fish of Group A were not subjected to stress (control group), while those of Group B were subjected to acute handling stress (3 times at 10 min intervals for 30 min).

From each fish biometric data and blood samples were collected. Fish of each group were no anaesthetised prior to blood sampling. Blood samples were collected immediately after capture (Group A) and immediately after the last stress administration (Group B) by venipuncture from caudal vein using a sterile plastic syringe (2.5 mL). Blood samples were transferred into a tube (Miniplast 0.5 mL; LP Italiana Spa, Milan, Italy) containing ethylenediamine tetraacetic acid (1.26 mg/0.5 mL) as an anticoagulant agent to evaluate haematological profile, blood glucose and lactate levels.

Haematological profile was assessed within 2 h from collection using a blood cell automatic counter HeCo Vet C (SEAC, Florence, Italy), which had been previously used to investigate haematological profile in *S. aurata* (Fazio *et al*., 2012b) and in other fish species (Faggio *et al*., 2013; Fazio *et al*., 2012a). Blood glucose and lactate levels were assessed immediately after blood sampling using the portable blood glucose (ACCU-Chek Active; Roche Diagnostics, Basel, Switzerland) and blood lactate analyser (Accusport, Boehringer, Germany). Unpaired t-test Student was applied to evaluate the differences of parameters studied between the two groups.

Results

No significant difference in biometric indexes between the two groups was found (Table 1). Red blood cells (RBC), haematocrit (Hct), haemoglobin (Hb), white blood cells (WBC), glucose and lactate levels ($P<0.05$) showed a significant increase in Group B in comparison with Group A, while mean corpuscular volume (MCV) statistically decreased ($P<0.05$) in Group B with respect to Group A (Table 2).

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Discussion

Aquaculture practices including intensive handling, crowding during most of the catching protocols, pre-slaughter and slaughter, could be a very traumatic time for the farmed fish, involving the onset of a stress status which can compromise the organoleptic, merchantable and sanitary quality of the final product. Stress is linked to a reduction of flesh quality and this is associated mainly with the pre-slaughter distress. In fact, the relative endocrine responses imply alterations before death starting processes of recall and intense consumption of glucose reserves which cause modifications of normal *post-mortem* processes and higher susceptibility to microbial attack. Stress during harvesting and death times plus relative endocrine response can hardly influence *post-mortem* biochemical processes such as the adenosine triphosphate degradation rate, *rigor mortis* onset and release, and freshness involution rate (Conte, 2004; Ashley, 2007; Poli, 2009).

The development of methods for monitoring metabolic indicators of stress in fish has obvious potential for improving husbandry protocols and post-harvest product quality. Haematological parameters as well as blood glucose and lactate levels are routinely used for the evaluation of physiological environmental and husbandry stressors in fish (Gabriel *et al*., 2011). In the present study significant higher RBC, Hct, Hb, WBC, glucose and lactate levels were found in Group B, while lower MCV levels was found in Group B compared to Group A.

Higher RBC, Hct, and Hb levels found in the stressed group may be due to splenic contraction. In fact, it is known that the spleen is a major storage organ for blood cells and it is known to contract in teleost fish during acute stress (Ruane *et al*., 2000). Contraction of the spleen results in the release of blood cells into the circulation and may account for the increase in erythrocytes. An enhancement in RBC, Hct and Hb levels following an acute stress was reported in most previous study (Olsen *et al*., 2008; Suski *et al*., 2007) and it was described as a strategy to improve blood capacity to carry oxygen under the high energy demand condition (Eslamloo *et al*., 2014). The MCV decrease in stressed group may indicate a release of smaller immature erythrocytes due to splenic contraction. According to several studies on stressed fish response (Puisford *et al*., 1994; Gabriel *et al*., 2011), the WBC increase in Group B confirms a stress condition and may also be caused by their migration from spleen to the blood circulation (Gabriel *et al.,* 2011). Although glucose and lactate levels' increase have been shown in fish exposed to chronic stress (Barcellos *et al*., 2009), the higher blood glucose and lactate levels found in this study in the stressed group could be caused by repeated acute stress affecting fish as chronic stress (Eslamloo *et al*., 2014). Higher level of plasma glucose following stress generally occurs in response to the release into the circulation of stress-induced hormones, mainly epinephrine and norepinephrine, triggering muscle or liver glycogenolysis and releasing glucose for the increased energy demands during and after stress (Eslamloo *et al*., 2014). Thus, the increase of glucose found in this study may be explained by a high demand of glucose by tissues of fish faced with repeated stress. The increased energy demands during and after stress also led to the increase in blood lactate. The increase of lactate is caused by anaerobic activity of muscles (Wang and Richards, 2011) and may be experienced in hypoxic stress conditions triggering instant glycolysis (Eslamloo *et al*., 2014). Plasma lactate has also been found to increase in other fish species 1 and 3 h after a single acute stress (Costas *et al*., 2011; Olsen *et al*., 2008; Suski *et al*., 2007).

The increase of anaerobic glycolysis in muscle due to higher energy mobilisation and utilisation likely to occur under stress condition implies an increase of lactate and a related decrease of muscle pH (Poli, 2009). When recovery of fish is not possible, for example when fish are slaughtered in a short time, the muscle pH will remain low and will further decrease due to the *post-mortem* glycolytic activity, thus compromising the quality of fish products (Poli, 2009).

Table 1. Mean values±standard deviation of biometric parameters obtained in Groups A and B.

Biometric parameters	*Sparus aurata* (Linnaeus, 1758) Group A (n=33)	Group B (n=33)
Fork length (cm)	19.64±0.95	18.96±1.10
Weight (g)	182.30±29.75	189.60±27.42
Condition factor (g/cm^3)	2.42±0.39	2.88±0.97

Table 2. Mean values±standard deviation of haematological, glucose and lactate data obtained in Groups A and B.

Blood parameters	*Sparus aurata* (Linnaeus, 1758) Group A (n=33)	Group B (n=33)
RBC (×10^6/μL)	2.74±0.13	3.35±0.40*
Hct (%)	49.83±2.51	56.98±3.40*
Hb (g/dL)	9.07±0.44	10.78±0.20*
WBC (×10^3/μL)	45.73±2.82	48.10±2.44**
TC (×10^3/μL)	104.75±36.73	110.23±30.11
MCV (fL)	181.60±1.99	171.20±6.87**
MCH (pg)	33.05±0.74	32.38±2.11
MCHC (g/dL)	18.34±0.61	18.92±0.87
Glucose (mg/dL)	124.60±32.45	254.00±34.46*
Lactate (mmol/L)	5.04±0.90	7.51±1.17*

RBC, red blood cells; Hct, haematocrit; Hb, haemoglobin; WBC, white blood cells; TC, thrombocyte count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration. **vs* Group A (P<0.0001); ***vs* Group A (P<0.05).

Conclusions

The results of this study show that acute handling stress affects some haematological parameters and secondary stress markers of *S. aurata* and suggest that the changes of these parameters are particularly useful for monitoring the stressful conditions of aquaculture production which affect not only animal welfare but also the quality of fish products.

References

Acerete L, Balasch JC, Espinosa E, Josa A, Tort L, 2004. Physiological responses in Eurasian perch (Perca fluviatilis L.) subjected to stress by transport and handling. Aquaculture 237:167-78.

Arends RJ, Mancera JM, Munoz JL, Bonga SEW, Flik G, 1999. The stress response of the gilthead sea bream (Sparus aurata L.) to air exposure and confinement. J Endocrinol 163:149-57.

Ashley PJ, 2007. Fish welfare: current issues in aquaculture. Appl Anim Behav Sci 104:199-235.

Barcellos LJG, Kreutz LC, Quevedo RM, Santos da Rosa JG, Koakoski G, Centenaro L, Pottker E, 2009. Influence of color background and shelter availability on jundiá (Rhamdia quelen) stress response. Aquaculture 288:51-6.

Conte FS, 2004. Stress and the welfare of cultured fish. Appl Anim Behav Sci 86:205-23.

Costas B, Conceição LEC, Aragão C, Martos JA, Ruiz-Jarabo I, Mancera JM, Afonso A, 2011. Physiological responses of Senegalese sole (Solea senegalensis Kaup, 1858) after stress challenge: effects on non-specific immune parameters, plasma free amino acids and energy metabolism. Aquaculture 316:68-76.

Eslamloo K, Akhavan SR, Fallah FJ, Henry MA, 2014. Variations of physiological and innate immunological responses in goldfish (Carassius auratus) subjected to recurrent acute stress. Fish Shellfish Immun 37:147-53.

Faggio C, Casella S, Arfuso F, Marafioti S, Piccione G, Fazio F, 2013. Effect of storage time on haematological parameters in mullet, Mugil cephalus. Cell Biochem Funct 31:412-6.

Fazio F, Faggio C, Marafioti S, Torre A, Sanfilippo M, Piccione G, 2012a. Comparative study of haematological profile on Gobius niger in two different habitat sites: faro lake and tyrrhenian sea. Cah Biol Mar 53:213-9.

Fazio F, Filiciotto F, Marafioti S, Di Stefano V, Assenza A, Placenti F, Buscaino G, Piccione G, Mazzola S, 2012b. Automatic analysis to assess haematological parameters in farmed gilthead sea bream (Sparus aurata Linneaus,1758). Mar Freshw Behav Phy 45:63-73.

Gabriel UU, Akinrotimi OA, Eseimokumo F, 2011. Haematological responses of wild Nile Tilapia Oreochromis niloticus after acclimatation to captivity. Jordan J Biol Sci 4:225-30.

Olsen RE, Sundell K, Ringø E, Myklebust R, Hansen T, Karlsen Ø, 2008. The acute stress response in fed and food deprived Atlantic cod, Gadus morhua L. Aquaculture 280:232-41.

Poli BA, 2009. Farmed fish welfare-suffering assessment and impact on product quality. Ital J Anim Sci 8:139-60.

Puisford AL, Lemair-gong S, Tomlinson M, Coiling Wood N, Glynn PJ, 1994. Effect of acute stress on the immune system of the Dab, Limanda. Comp Biochem Phys C 109:129-39.

Ruane NM, Nolan DT, Rotllant J, Costelloe J, Wendelaar Bonga SE, 2000. Experimental exposure of rainbow trout Oncorhynchus mykiss (Walbaum) to the infective stages of the sea louse Lepeophtheirus salmonis (Krøyer) influences the physiological response to an acute stressor. Fish Shellfish Immun 10:451-63.

Suski CD, Cooke SJ, Danylchuk AJ, Connor CM, Gravel M-A, Redpath T, Hanson KC, Gingerich AJ, Murchie KJ, Danylchuk SE, Koppelman JB, Goldberg TL, 2007. Physiological disturbance and recovery dynamics of bonefish (Albula vulpes), a tropical marine fish, in response to variable exercise and exposure to air. Comp Biochem Phys A 148:664-73.

Wang Y, Richards JG, 2011. Hypoxia: anaerobic metabolism in fish. In: Farrell A, ed. Encyclopedia of fish physiology. Academic Press, Waltham, MA, USA, pp 1757-63.

Wells RMG, Pankhurst NW, 1999. Evaluation of simple instruments for the measurement of blood glucose and lactate, and plasma protein as stress indicators in fish. J World Aquacult Soc 30:276-84.