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Hematological Recovery in *Sparus aurata* after Bleeding. A Time Course Study

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To determine the hematological recovery after blood loss in gilthead sea bream juveniles, an experimental anemia was induced by caudal blood extraction. Seventy fish were used for experimental hemorrhage and another 35 were used as controls. Blood samples were taken after 0.5, 1, 2, 4, 8, 16 and 32 days in both control and anemic groups. After bleeding, besides a reduction in the direct hematic constants, a peak in the mean cell volume value, together with a decrease in mean cell hemoglobin concentration after bleeding, suggested erythrocyte swelling. The increase in MCH suggested the occurrence of hyperchromic erythrocytes. Recovery of RBC number started between 2 and 4 days after bleeding and seemed to be related to erythroblast release to circulation. Total recovery was completed after 8 days of bleeding. The recovery patterns for hematological parameters of sea bream are discussed in relation to applied work on this species.

Key words: Anemia, Bleeding, Hematology, Red blood cells, Sea bream, *Sparus aurata*, Stress.

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Different techniques, including dorsal and ventral aorta or hepatic vein cannulation (8, 21, 23), removal of gonads or spleen (1, 24) often involve blood loss. HOUSTON (12) reported losses of up to 0.5 ml when introducing cannulae to the dorsal aorta. Duthie and Tort (8) showed a 20 % decrease in both hematocrit and RBC after dorsal aorta cannulation in the dogfish *Scyliorhinus canicula.* An increase in MCV was reported in gilthead seabream (AISSAOUI *et al,* unpublished observations) and in dogfish (29) after cannulation. In addition, some physiological monitoring studies require a repeated blood sampling in short periods of time. This involves a consistent loss of significant blood volume, particularly when working on small fish. However, most of these works evaluated the impact of blood loss after a short period of time, and less work has been done on longer term effects of blood losses (4) or on time-course recovery after blood sampling. Thus, most studies started physiological recordings after 24 hours of surgical operations, taking the hematocrit as the only indicator of physiological recovery. Reported data on the effects of bleeding on hematological parameters included changes in the abundance of RBC in peripheral blood (16) or erythrocyte swelling (26).

The rapid increase of gilthead sea bream farming in the Mediterranean region, and particularly in Spain and Greece, underlines the need for studies on biological parameters such as hematology and their dynamics after handling. However, only few works have concentrated on this species (6, 7, 17)

Therefore, the aim of this work was to determine recovery lines for some selected hematological parameters after bleeding gilthead sea bream juveniles. Since most monitoring procedures require blood sampling by discrete syringe punctures, rather than most stressful chronic cannulation, this work has concentrated on recovery after bleeding.

Materials and Methods

One hundred and five fish from a local bream farm (ADSA) were stocked in 21 cylindrical fibre-glass 100 l tanks, supplied with a continuous flow (1.5 l/min) of pumped seawater. Natural photoperiod during the experiment was 10:14 L/D and water temperature ranged between 17.8-18.1 °C. Five fish (88.40 ± 12.67 g B. W.) were stocked per tank, and kept for 20 days to allow for acclimation to the tank. Fish were fed twice a day with commercial dry pellets (Troutvit: Trouw España S. A., Cojobar/Burgos, Spain) at a rate of 3 % body weight per day. Once acclimation to tanks was completed, all fish were exposed to a handling process: the anesthetic (chlorobutanol: methyl propanol-3-chlorine, 200 mg/l for 4 min) was added directly to the tanks to avoid hemoconcentration produced by the capture of the fish with net prior to anesthesia (24). Then, from every fish in 14 of the tanks, fish were kept out of water for a maximum time period of one minute for 1 ml of blood extraction, which represents about 35 % of the total body blood volume (30). The other 7 tanks were considered as the control group. Next blood samplings were performed after 0.5, 1, 2, 4, 8, 16 and 32 days, and consisted of the extraction of 1 ml from each individual of two experimental and one control tanks.

Blood was sampled with plastic 1 ml syringes in a single extraction to avoid the effects of two consecutive extractions on hematocrit (11) and was extracted by puncture in the caudal vessels and lithium heparin was used as anticoagulant. Hematocrit (microcentrifugation for 5 min) and hemoglobin (Boehringer-Mannheim-test combination kit) measures were per-

formed at once. The number of erythrocytes (RBC) where determined using optical microscopy and the hematological calculations, i. e. mean cellular hemoglobin concentration (MCHC), mean cellular volume (MCV), and mean cellular hemoglobin (MCH) were calculated from the basic hematological variables (9).

All the data were subjected to one-way analysis of variance (ANOVA). Differences between means were compared by the Tukey test at a 95 % interval of confidence ($P < 0.05$). Kruskal-Wallis tests were used for non-parametric data.

Results

No mortality was observed along the experimental period. Initial values obtained for hematocrit, hemoglobin, RBC, MCHC, MCV and MCH (table I) were used as a reference to study the dynamic of hematological parameters. Control fish did not show significant differences in any parameter compared to initial values, except for a slight increase in the RBC number 12h after handling (fig. 1).

The loss of 35 % of the total blood volume in gilthead sea bream produced a significant decrease in the hematocrit (34.28 %), which was already detected 12 h after bleeding (fig. 1). The values remained significantly different from both controls and the initial value, along 4 days after bleeding, whereas similar values compared to the initial ones were found from 8 days after bleeding. The blood loss also produced a decrease in hemoglobin content (49.02 %), being significantly lower at 12 h sampling. Recovery took place from day 4 and values from day 8 onwards were similar to the initial ones.

The RBC number abruptly decreased after bleeding (55.56 %), the values being significantly lower 12 h after sampling. From this point, a slow but gradual recovery was observed (fig. 1).

MCHC values were used as an indicator of erythrocyte swelling (28). A significant decrease is observed after 24 hours in

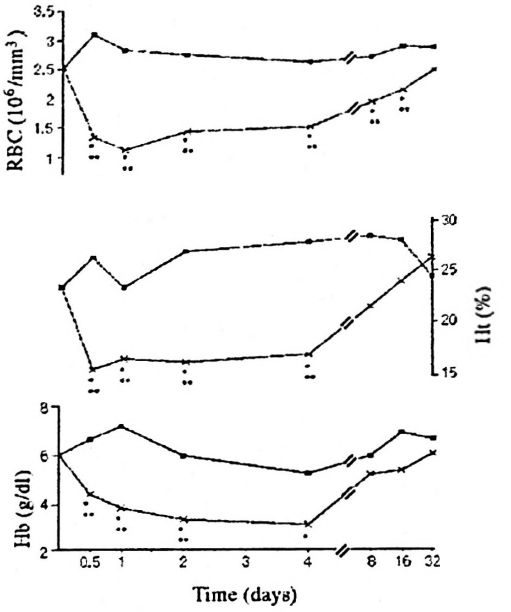

Fig. 1. *Changes of hemoglobin value (Hb), hematocrit (Ht) and number of erythrocytes per mm^3 of blood (RBC).*
Control group (■); Hemorrhagic group (x). *P < 0.05 versus initial values, **P < 0.05 versus control group.

Table I. *Initial value of Hematological parameter for gilthead sea bream juveniles.*

Parameters	(mean ± SD; n=50)
Hematocrit (%)	23.3934 ± 3.7362
Hemoglobin (g/dl)	5.9582 ± 0.9909
RBC count (x10^6 cell/mm^3)	2.5190 ± 0.3437
MCHC (g/dl)	24.9823 ± 4.1881
MCV (μ^3)	90.9250 ± 12.9000
MCH (pg)	23.6530 ± 3.7700

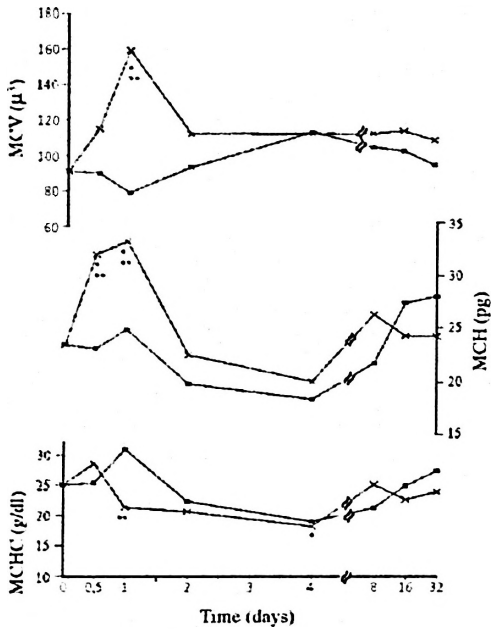

Fig. 2. *Changes of mean cellular hemoglobin concentration (MCHC), mean cellular hemoglobin (MCH) and mean cellular volume (MCV).*
Legend as in figure 1.

hemorrhagic fish, although the difference is not significant compared to the initial values of the same group. In addition, MCH values were significantly higher 1 h and 2 h after bleeding with a progressive decrease down to day 2 after bleeding, when there were no significant differences with the control group or initial values. MCV values were significantly higher at 24 h. From this time, values gradually recovered, and no significant differences were observed 2 days after bleeding compared to initial and control values (fig. 2).

Discussion

Bleeding produces in fish a general reduction of hematological values. Hematocrit values showed a trend towards stability (around 16 %), between 12 h and 4 d after bleeding. MURAD and HOUSTON (22) reported a similar trend in hematocrit for goldfish (*Carassius auratus*) exposed to a hemolytic agent (phenylhydrazine HCl) by immersion, although recovery time-periods are different for the two fish species. CAIRNS and CHRISTIAN (4) found a continuous decrease in hematocrit after daily blood extractions from rainbow trout (*Oncorhynchus mykiss*) although this could be explained as due to a continued blood loss. Hemoglobin and RBC values for gilthead sea bream exposed to anemia induced by bleeding showed decreases as described for rainbow trout (*O. mykiss*) after loss of 15 % body blood volume (16). In the same way, SCHLINDER and DE VRIES (26) reported a decrease in erythrocyte number after bleeding to about 1/3 in carp (*Cyprinus carpio*).

Stressful handling and anesthesia produce polycythemia on circulating erythrocyte population (32). No effects of the anesthetic were observed in sea bream. Fish reached stage 4 of anesthesia, i.e. loss of the reflex activity and response to strong stimuli (27) without any mortality. By contrast, higher concentrations of the same anesthetic do not induce the same stage in cod (*Gadus morhua*) (18). In our study, fish subjected to initial handling without bleeding (control group) showed a peak in RBC 12 h after handling which could be due to the effect of handling on the red blood cell population (32). On the other hand, the lowest RBC value appeared 24 h after bleeding while the 12 h after bleeding value seems to be a result of the same effect, although these values were not significantly different. The combination of blood extraction plus handling produces an increase in MCH values in fish shortly after bleeding. This effect has been attributed to the presence of hyperchromic erythrocytes for carp (*C. carpio*) and rainbow trout (*O. mykiss*) under sim-

ilar treatments (13, 26). In the present study, only fish subjected to bleeding showed a peak in MCH values 12 h after bleeding, in contrast to those fish subjected to previous handling without bleeding, which did not show significant differences with the initial MCH value.

Erythrocyte swelling has been associated to stress in fish (28). The peak in MCV value, as well as the significant decrease in MCHC values after bleeding, indicate a marked erythrocyte swelling caused by anemia induced by bleeding. Hypoxia or forced swimming lead to the release of plasma catecholamines (3, 19, 31), which give rise to different effects in order to counteract hypoxia, including vessel constriction in most arteries of teleosts (11), decrease of hemodynamic resistance in gills (2), or increase in aortic pressure (20). In addition, catecholamines exert an adrenergic action on the red cell membrane, leading to a switch in the Na^+-H^+ exchange mechanism, plasma alkalinization and inflow of Na^+ and Cl^- with the subsequent red cell osmotic swelling (5, 25, 28). In the present experiment, hypoxia due to anesthetic and handling plus oxygen deficit due to anemic blood, seemed to cause swelling of erythrocytes in the hemorrhagic fish. However, another factor, i.e. the release of juvenile blood cells, could also help produce these changes in volume. The production of new red blood cells (erythroblasts) would correspond to day 4 after bleeding (14). These new red blood cells have been reported to have less than half of hemoglobin content than mature cells (15, 26).

Recovery signs in RBC values appeared between 2 and 4 days after bleeding in hemorrhagic fish, which can be related to erythroblasts release in circulating blood. As these cells have a reduced content in hemoglobin, total hemoglobin in blood remains at low values, despite an RBC and a hematocrit value increase. Nevertheless, the direct measurement of total blood hemoglobin gives a significant indication of the hematological dynamics and oxygen needs. Thus, most fish species possess polymorphic Hb systems and, as said before, the cell population may well be a mixture of juvenile and mature forms. In such a situation the total hemoglobin should be a better indicator than hematocrit or erythrocyte number. In the present study, the total hemoglobin significantly and abruptly decreases at the first sample, but values do not immediately recover. They remain low for 4 days, unless other hematological indicators appear. This seems to support the fact that new cells released to the blood stream would have less hemoglobin. The same conclusion is suggested by the fact that the MCV increase is much more pronounced than the MCH one. The source of these cells could be diverse, i.e. release from splenic or hepatic reservoirs, accelerated erythropoiesis or maturation, or cell division.

Concerning recovery, the results from this work show that gilthead sea bream juveniles with 35% blood loss, recover initial values at day 8. This agrees with data obtained by Lane (14) for rainbow trout (*O. mykiss*), who reported a recovery time period of 9 days after two blood extractions of 12 % of the total volume. However, RBC completely recovered after 32 days, showing a different trend from the other parameters. SCHINDLER and DE VRIES (26) also reported a long RBC recovery time of 4 weeks after bleeding in carp (*C. carpio*). Concerning the process of recovery, a progressive approach to initial values is observed in most parameters. This type of dynamics could be explained by the fact that a loss of volume of approximately 35 % is strong enough to induce different types of responses, some directed to meet physiological demands of tissues (respiratory,

cardiovascular) or restore volume (kidney, osmoregulatory hormones) and others to restore blood cells. While the former are rapid responses, the latter may take some days (14). Therefore, hematological values will recover initial figures progressively and not abruptly, even more when one of these processes is the RBC maturation itself, which is a progressive rather than an abrupt change.

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En peces se sabe poco sobre los efectos a largo plazo asociados a pérdidas de sangre, o sobre la recuperación después de manejos que implican extracción de sangre A tal objeto, en 70 ejemplares juveniles de dorada se determinan algunos parámetros hematológicos tras inducción de anemia por extracción de sangre. Las muestras se toman por punción caudal a los días 0, 0,5, 1, 2, 4, 8, 16 y 32. Se observa un descenso en el número de eritrocitos, hemoglobina y hematocrito, y un aumento en el volumen celular medio y hemoglobina celular media. Se observa un pico en el volumen celular medio, junto con un descenso en la concentración media de hemoglobina corpuscular después de la extracción de sangre. La evolución de estos parámetros sugiere "hinchazón" de los eritrocitos causada por la anemia. Un incremento en hemoglobina corpuscular media sugiere la presencia de eritrocitos hipercrómicos. Los signos de recuperación en el número de eritrocitos comienzan a observarse entre los días 2 y 4 después de la pérdida de sangre, y parecen estar relacionados con la aparición de eritroblastos en la circulación sanguínea. La recuperación total es evidente a partir del día 8 de la extracción. Se estudian y discuten las pautas de recuperación de los diferentes parámetros.

Palabras clave: Anemia, Extracción de sangre, Hematología, *Sparus aurata,* Estrés.

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