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Research Article

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Characterization of Blood Cells, Hematological and Biochemical Parameters in *Diplodus noct* from the Red Sea

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Abstract

The main aim of the present study was to obtain a basic knowledge of the hematology and biochemistry of the Red Sea seabream *Diplodus noct*. The samples were collected from the Red sea at Hurghada, Egypt. Baseline values for hematological parameters including Red Blood Corpuscles (RBCs), Hematocrit (HCT), Hemoglobin (Hb), Mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH) and mean cell volume (MCV) and biochemical parameters including AST, ALT, glucose, total protein and urea were established. Erythrocytes, thrombocytes and three types of leucocytes, lymphocytes, neutrophils and eosinophils, were distinguished, characterized and measured. The morphological and cytochemical aspects of peripheral blood cells of *Diplodus noct* were studied by light microscopy. This investigation may be helpful as a tool to monitor the health status of *Diplodus noct* and will grant early detection of clinical pathology.

Keywords

Seabream; Hematology; Biochemistry; Blood cells; Aquaculture; Health

Introduction

Interest in fish as laboratory animals has increased considerably in the last few decades [1]. There are several investigations on their physiology, biochemistry and pathology [2,3].

It has been reported that, hematological indices and blood biochemistry parameters are important parameters for health status of farmed animals and evaluation of fish physiological and pathological status [4,5]. Changes in those parameters depend on the fish species, age, sex and health [6-9].

Normal ranges for various hematological and biochemical parameters in fish have been established by different investigators in fish physiology and pathology [5,9-16].

Although, structural features of fish blood cells are one of the best indicators of body health; very little attention has been paid to such investigations [1,9,10,17-20]. The studies on this topic to date have posed numerous problems derived from both the nomenclature and the techniques used. Hematological nomenclature referring to higher vertebrates has been applied to fish based on morphological similarities alone and only in a few cases a different nomenclature has been minted for fish and as a consequence, it is difficult to compare the results available from different studies [21].

The studies of Oria [22], Phillips Jr [23] and Black et al.[24] in the haemoichthyology acted as pioneers; they established a comparison of the hematological aspect among several species of fish. In the following decades, many studies have been performed to establish the hematology index of teleost fish [25,26]. Studies on the erythrocyte lineage cells and morphology have been carried out [25,27,28]. Other studies have demonstrated a special interest in the leucocytes of fish with regard to their morphological observations and their absolute values, considering a great diversity of morphological aspects to the same type of leucocyte [25,28,29].

Cytochemical staining is useful tool for identification of cellular lineage and may suggest cell function and it is also critical for identifying immunological cell types associated with pathological processes [8,30,31]. Many studies have been done using cytochemical aspects of blood cells in different species of fishes [1,9,10,17,20].

The present study aimed to investigate the normal values of hematological and biochemical parameters and the morphological and cytochemical characteristics of the cellular blood components of the Red Sea seabream (*Diplodus noct*) from the Red Sea, Egypt. This investigation may be helpful as a tool to monitor the health status of this and other related fish species. We suggest that the hematological studies on fishes are of great significance due to the increasing emphasis on pisciculture and greater awareness of the pollution in aquatic ecosystem.

Materials and Methods

Fish collection

A total number of 15 adult male specimens (Figure 1a) of the Red Sea seabream (*Diplodus noct*) with a length of (17 ± 0.6 cm, range: 14-21) and a weight of (68 ± 5.05 gm, range: 50-95) were captured from the Red Sea at the National Institute of Oceanology and Fisheries (NIOF), Hurghada, Egypt (Figure 1b), in April 2014. Netting was the main fishing method used to collect the fish.

Hematological and biochemical analyses

The specimens were anaesthetized with clove oil, 75 mgl^{-1} [32], and the peripheral blood was collected from severed caudal peduncle into heparinized tubes. These blood samples were used for determining erythrocyte count (RBC's) using hemocytometer [33]. Hemoglobin level (Hb) was determined by using suitable kits (Diamond Diagnostics, Egypt) according to Stoskopf [34]. Hematocrit value (Hct) was calculated according to the formulae mentioned by Stoskopf [34]. Mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), and mean cell volume (MCV) were calculated using the formulae mentioned by Dacie and Lewis [33].

$$MCHC\ (\%) = Hb/Hct \times 100$$

$$MCH\ (p.g) = Hb/RBC \times 10$$

$$MCV\ (\mu m^3) = Hct/RBC \times 10$$

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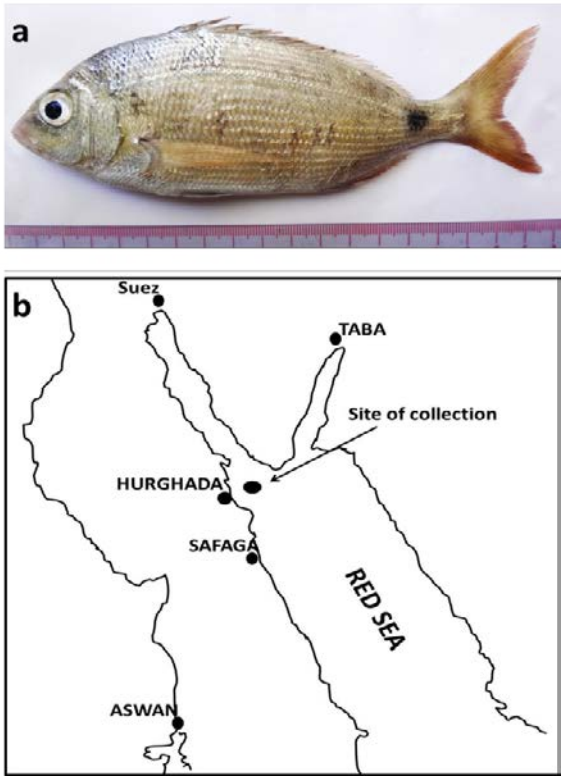

Figure 1: (a) Fish of Study; Diplodus noct (b) The Red Sea map showing the site of collection.

Some other blood samples were collected and left to coagulate for 15–20 min at 4°C prior to centrifugation for 20 min at 3,000 rpm to separate serum. The fresh serum was subjected to biochemical analysis. Serum glucose (mg/dl) and total protein (g/dl) were determined, using assay kits supplied by (Spectrum Diagnostics, Egypt). Urea level (mg/dl) was determined using assay kits supplied by (Diamond Diagnostics, Egypt). Activities of aspartate aminotransferase (AST, U/I) and alanine aminotransferase (ALT, U/I) were determined colorimetrically using assay kits (Spectrum Diagnostics, Egypt) according to Reitman and Frankel [35]. The samples were measured by spectrophotometer (Ultroscopec 3100 Pro).

Morphological and cytochemical analysis of blood cells

Blood smears were prepared and fixed in absolute methanol for 3 min at room temperature after drying. The blood cell size was established by measuring mean length and width of the cells and their nuclei on micrographs with Image J 1.48v software.

For the morphological analysis, blood smears were stained with Hematoxylin and Eosin (H&E) [36]. For the cytochemical analysis, the blood smears were stained with the Periodic Acid-Schiff (PAS) [37] and counterstained with hematoxylin for glycogen demonstration and mercuric bromphenol blue (BB) method for basic protein [38]. Blood smears were examined using Ziess microscope and photographed using a digital colored video camera (OMAX, 14Megapixel).

Statistical analyses

All data obtained were presented as mean ± SE (Std. Error of Mean). One way ANOVA (analysis of variance) between means and differences in means were considered significant when $p<0.05$ using statistical program, version 16 (SPSS, 1998).

Ethical statement

All experiments were carried out in accordance with the Egyptian laws and University guidelines for the care of experimental animals. All procedures of the current experiment have been approved by the Committee of the Faculty of Science of Assiut University, Egypt.

Results

Hematological and biochemical parameters

Hematological and biochemical parameters values of *D. noct* are listed in Table 1.

Blood cells characterization

In relation to the morphological elements of peripheral blood of *Diplodus noct*, it was possible to identify the following cells: erythrocytes, thrombocytes, neutrophils, eosinophils and lymphocytes. The criteria used to define the nomenclature of these cells were based on the morphological aspect of the nucleus and the distribution pattern of cytoplasmic granules [1].

Red blood cells: Biconvex erythrocytes are the predominant cell type found in *Diplodus noct* blood. Under light microscope with hematoxylin and eosine staining they are predominantly ellipticalin shape and contained central, blue-violet-stained nuclei that generally accompanied the format of the cells and homogeneous and light-pink-stained cytoplasm (Figure 2a).

White blood cells: Lymphocytes are spherical cells, with little basophilic cytoplasm and frequently show cytoplasmatic blebs. Their nuclei are spherical, purple and fill most of the cell. According to cell size, lymphocytes are classified into small (Figure 2b) and large lymphocytes (Figure 2c).

Neutrophils are usually large round or irregular outlined cells. Their nuclei, stained purple, are usually ovoid, kidney-shaped, bilobed and eccentric. The cytoplasm contains very small pink neutrophilic granules and small vacuoles (Figure 2d).

Eosinophils (acidophils) are round and medium sized cells. Their nuclei are round, generally eccentric, but occasionally in a central position. The cytoplasm is rich in large eosinophilic granules (Figure 2e).

Thrombocytes are present in different shapes in blood smears; fusiform to spindle, round, elliptical, tadpole-like or oval-shaped with a nucleus following the shape of the cell. Spiked thrombocytes with a long, curved and terminal process are found but uncommon. The cytoplasm of thrombocytes is light-pink or hyaline. Thrombocytes appear as single cells or occur in clusters with 2–30 cells (Figure 2f-k).

Size of the cells: The blood cell sizes of *D. noct* are listed in Table 2.

Cytochmistery of blood cells

The results of the cytochemical reactions in different blood cells with different stains are summarized in Table 3. The results show that only neutrophils (Figure 3c), and eosinophils (Figure 3d) are stained by PAS while erythrocytes (Figure 3a), eosinophils (Figure 3a) and neutrophil (Figure 3b) are stained with bromophenol blue. Lymphocytes (nongranular mononuclear cells) and thrombocytes are stained neither by PAS nor by BB.

Table 1: Hematological and biochemical parameters of *Diplodus noct*, n=6.

Hematological parameters	Values (Mean ± SE)	Biochemical parameters	Values (Mean ± SE)
RBC's (× 10^6/mm^3)	3.3 ± 0.03	AST (GOT)(IU/l)	39 ± 1.87
Hb (g/dl)	6.8 ± 0.06	ALT (GPT)(IU/l)	45.3 ± 0.4
Ht (%)	19.8 ± 0.14	Glucose(mg/dl)	124.3 ± 0.33
MCV (μm^3)	60 ± 1.04	Total protein(g/dl)	7.8 ± 0.05
MCH (p.g)	20.6 ± 0.34		
MCHC (%)	34.3 ± 0.25		

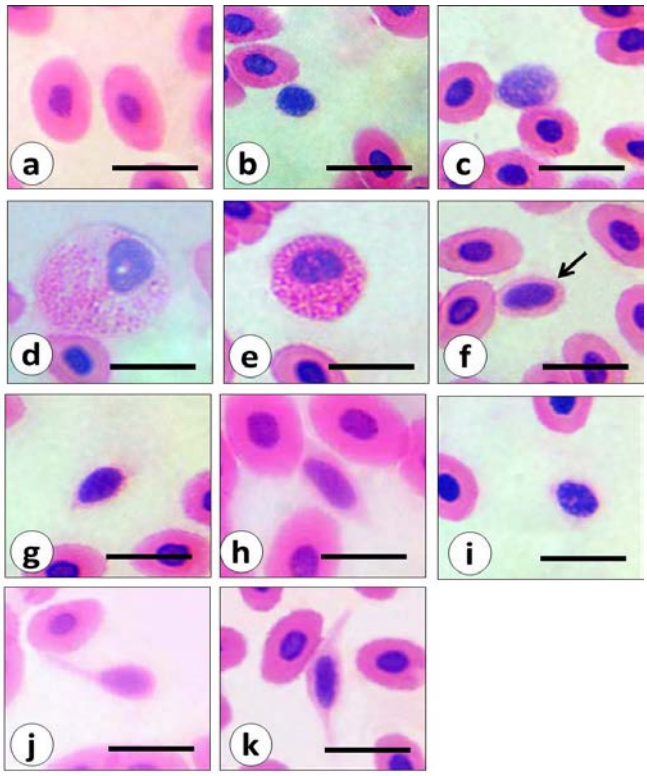

Figure 2: The microstructure of peripheral blood cells of *Diplodus noct* (Hematoxylin& Eosin) (a) erythrocytes; (b) small lymphocyte; (c) large lymphocyte; (d) neutrophil; (e) eosinophil; (f) elliptical thrombocyte (arrow); (g) oval-shaped thrombocytes; (h) fusiform thrombocyte; (i) round thrombocyte; (j) tadpole-like thrombocyte; (k) spiked thrombocytes. Scale bar = 10 µm.

Discussion

Previous studies have been reported that, interpretation of hematological parameters in fish is quite difficult because variations in the blood indices are caused by internal and external factors [13].

Hematological parameters (RBCs count, Hb, MCV, MCHC, Hematocrit) used as indicators for anemia in fish [13,39]. In the present study, the mean erythrocyte count obtained for *D. noct* was 3.3 ± 0.03(×10^6/mm^3) and that result agreed with the value reported for the yellowfin seabream, *Acanthopagrus latus* [12]. Also, it was similar to the mean erythrocyte counts of *Dicenrarchus labrax* L. [40] and *Cichlasoma dimerus* [9], but it was higher compared to *Anguilla anguilla* [41]. Mean Hb value for *D. noct* in the present study was 6.8 ± 0.06 (g/dl) and that agreed with the mean Hb value of the yellowfin seabream, *Acanthopagrus latus* [12] but it was higher than the values reported for *Heterotis niloticus* [42] and *Parachanna obscura* [43] and it was lower than the value reported for *Synodontis membranacea* [44]. Hct mean value for *D. noct* in the present study was 19.8 ± 0.14 (%) and this result agreed with that reported for *Parachanna obscura* [43] but it was lower than that reported for the yellowfin seabream, *Acanthopagrus latus* [12]. MCV mean value for *D. noct* in the present study was 60 ± 1.04 (μm^3) and it was higher than the MCV mean values reported for *L. calcarifer, A. subrostratus, P. canius* and *S. javus* [14] but it was lower than that reported for the yellow fin seabream, *Acanthopagrus latus* [12]. Also, MCH mean value for *D. noct* was 20.6 ± 0.34 (p.g) and that result agreed with the value reported for the yellowfin seabream, *Acanthopagrus latus* [12] and it was similar to that of *Mugil cephalus* [11] but it was lower than the MCH mean value reported for *Parachanna obscura* [43]. MCHC mean value for *D. noct* was recorded as 34.3 ± 0.25 (%) in the present study and it was similar to that of *Capoeta trutta* [45] and *Parachanna obscura* [43] but it was higher than that reported for the yellowfinseabream, *Acanthopagrus latus* [12].

In the present study, the serum glucose mean value of *D. noct* was 124.3 ± 0.33 (mg/dl) and it was similar to that reported for the hybrid tambacu [8] but it was higher than the values reported for *L. calcarifer*, *M. cephalus*, *C. chanos*, *A. subrostratus*, *P. canius* and *S. javus* [5,14] and it was lower than the values reported for *Dicentrarchus labrax* and *Sparus aurata* [11]. The total protein mean value of *D. noct* was 7.8 ± 0.05 (g/dl) and it was higher than the corresponding of *P. mesopotamicus*, *C. macropomum* and hybridtambacu [15]. AST mean value of *D. noct* in the present study was 39 ± 1.87(IU/l) and it was similar to that obtained for *Clarias gariepinus* [46] but it was lower than the value obtained for *Oreochromis niloticus* [47]. ALT mean value of *D. noct* in the present study was 45.3 ± 0.4 (IU/l) and it was similar to the mean value reported for *Oreochromis niloticus* [47] but it was higher than that obtained for *Clarias gariepinus* [46] and it was lower than the mean value reported for *Liza klunzingeri* [48].

Stained blood films in the present study revealed that the circulating population of blood cells in *D. noct* consists of erythrocytes, neutrophils, eosinophil, lymphocytes, and thrombocytes. In contrast with the present study, basophils, monocyte/macrophage and plasma cells are observed in the blood of the gilthead Seabream *Sparus aurata* [18]. In the present study, the mature erythrocytes of *D. noct* showed an average size larger than other fish species such as *Cichlasoma dimerus* [9] but they were smaller than some fishes such as

Table 2: Size ofdifferent blood cells (length × width) of *Diplodus noct*, n=6.

Cells	Cell size(length × width; μm^2)	Nucleus size(length × width; μm^2)
Erythrocytes	(10.28± 0.15) × (5.88± 0.09)	(3.74 ± 0.09) × (2.47 ± 0.05)
Small lymphocytes	(4.31 ± 0.19) × (4.27 ± 0.21)	(3.65 ± 0.17) × (3.55 ± 0.19)
Large lymphocytes	(6.32 ± 0.24) × (5.09 ± 0.14)	(4.56 ± 0.15) × (4.43 ± 0.18)
Neutrophils	(13.85 ± 0.38) × (12.37 ± 0.47)	(4.84 ± 0.34) × (6.24 ± 0.32)
Eosinophils	(10.08 ± 0.43) × (9.77 ± 0.35)	(4.35 ± 0.19) × (4.98 ± 0.30)
Thrombocytes	(7.0 1± 0.71) × (4.15 ± 0.15)	(4.96 ± 0.25) × (2.73 ± 0.10)

Table 3: Cytochemical staining of blood cells of *Diplodus noct*. Periodic acid-Schiff staining (PAS) and bromophenol blue staining (BB).

Cells	PAS	BB
Erythrocytes	-	+++
Thrombocytes	-	-
Lymphocytes	-	-
Neutrophils	+++	++
Eosinophils	+	++

Negative (-), weakly positive (+), positive (++), highly positive (+++)

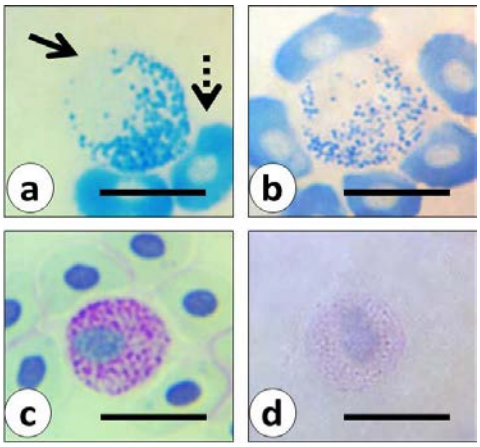

Figure 3: Cytochemical staining of blood cells of the peripheral blood of *Diplodus noct*. Bromphenol blue method demonstrates the positive staining in the cytoplasm of erythrocytes (dotted arrow) and the granules of eosinophil (solid arrow) (a) and neutrophil (b). PAS method + Harris Hematoxylin demonstrate the presence of glycogen in the cytoplasm of neutrophil (c) and eosinophil (d). Scale bar = 10 µm.

Schizothorax prenanti [17], *Thunnus maccoyii* [49], *Acipenser sinensis* [50] and *Gymnocypris eckloni* [51]. Their morphological features were similar to those of *H. littorale* [19] and *S. prenanti* [17].

In the present study, the thrombocytes showed different morphological shapes, from fusiform to spindle, tadpole-like, spiked or oval-shaped cells, which agree with reports on other fish species [17,18,49,51-53]. However, the predominant types of thrombocytes were variable among fish species as oval and spike-shaped thrombocytes in *A. sinensis* [52], fusiform in four freshwater teleosts [31], and oval in, *Carassius auratus* , *Acipenser transmontanus* [20] and *G. maculatum* [53], in the present study, elliptical thrombocytes were the most frequent just as those of *G. eckloni* [51]. Although, some authors failed to distinguish thrombocytes from lymphocytes [21,54], in the present study we differentiated between them by well-stained preparations as their light-pink or hyaline cytoplasm (thrombocytes), in contrast with bluish cytoplasm covering a narrow area around the nucleus of the lymphocyte. The thrombocytes size of *D. noct* was similar to that of *G. eckloni* [51], *S. prenanti* [17] but it was smaller than the thrombocytes of *A. sinensis* [50].

Morphological shape of the lymphocytes which was observed in our study was similar to fish species reported by other workers[1,9,17,18,51]. Their size was similar to the lymphocytes of *Thunnus maccoyii* [49] but it was smaller than those of *G. eckloni* [51], *A. sinensis* [50] and S. *prenanti* [17].

We didn't identify any of monocyte-macrophages in the blood of *D. noct* studied and this agrees with other researches [55,56] whereas they have been reported by others [9,17,51].

In the present study, two kinds of granulocytes (neutrophils and eosinophils) were identified in the Red Sea seabream peripheral blood. This result agreed with some other fish species such as *T. maccoyii* [46], *A. sinensis* [52], *C. dimerus* [9], *B. splendens* [7] and *G. eckloni* [51]. But unlike *H.littorale* [19] with three kinds of granulocytes (neutrophils, heterophils and eosinophils) and *S. prenanti* [17] with only one kind of granulocyte (neutrophils).

The neutrophils size of *D. noct* was similar to those of *G. eckloni* [51] but it was larger than those of *T. maccoyii* [49] and *S. prenanti* [17] and it was smaller than those of *A. sinensis* [50]. It's morphology was similar to that of *A. sinensis* [50], *H.littorale* [19] and *S. prenanti* [17]. The eosinophile size of *D. noct* was similar to those of *T. maccoyii* [46] but it was smaller than those of *A. sinensis* [50] and *G. eckloni* [51]. It's morphology was similar to that of *T. maccoyii* [49] and *H. littorale* [19].

Cytochemical staining is particularly useful for the study of cellular lineage where the presence of large stores of glycogen in cells is related to their particular function [31]. In the present study, PAS-positive staining in neutrophils of *D. noct* corroborated the findings in other fish species [10,17,57]. Also, eosinophils were PAS positive just like those of *A. transmontanus* [20], *T. maccoyii* [49], *A. ocellatus* [31] and *A. gigas* [58].

On the other hand, *D. noct* lymphocytes were PAS negative and this result matches with others in *O. mykiss*, *O. kisutch*, *A. transmontanus*, *M. saxatulis* [20], *H. littorale* [19], *A. bimaculatus*, *A. ocellatus*, *H. malabaricus*, *A. nobilis* [31], *I. punctatus* [8], *S. lima* [10] and *T. maccoyii* [49], in contract with others as positive in *C. auratus* [20], *O. niloticus* [1], *M. peelii* [30] and *S. Prenanti* [17]. Also, thrombocytes of *D. noct* were found to be PAS negative just like those of *M. saxatilis, O. mykiss and O. kisutch* [20] and *A. bimaculatus* [31]. On the other hand, in thrombocytes of *I. punctatus, A. transmontanus* and *C. auratus* [20], *H. littorale* [19], *A. ocellatus*, *H. malabaricus* and *A. nobilis* [31] and *S. lima* [10] the PAS reaction was positive.

In the present study, neutrophils, eosinophil and erythrocytes of *D. noct* showed positive staining for bromophenol blue, like the eosinophils of *S. maxillosus* [59], *O. niloticus* [1]and *A. gigas* [58] and neutrophils and eosinophils of *S. lima* [10]. Ueda et al., 2001[1] stated that, the positive staining observed in the eosinophilic granules in particularly, may be due to the presence of antilarval substance-like referred classically as major basic protein (MBP) [60] or as eosinophilic cationic protein (ECP) [61] for human. On the other hand, lymphocytes were negative just like those of *S. lima* [10]. Thrombocytes of *D. noct* were negative as well.

Conclusion

In conclusion, the present study provides a contribution to the knowledge of hematological and biochemical parameters in addition to the characteristics of the blood cells of the Red Sea seabream *Diplodus noct* under normal conditions in comparison with similar or different species.

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