# Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes)

G. Rey Vázquez *, G.A. Guerrero

*Laboratorio de Embriología Animal, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, C1428EHA Buenos Aires, Argentina*

## Abstract

The aim of the present study was to obtain a basic knowledge of the hematology of *Cichlasoma dimerus*. The morphological features of blood cells were described according to the observations made by light and electron microscopy. Erythrocytes, thrombocytes and four types of leucocytes: lymphocytes, monocytes, heterophils and eosinophils, were distinguished and characterized. Thrombocytes are the most abundant blood cells after erythrocytes and are recognized easily from lymphocytes by morphological features and size. Heterophils and eosinophils are PAS positive.

Hematological indices (RBC, WBC, PCV, Hb, MCV, MCH, MCHC and leucocyte differential count) were measured in one blood sample from 30 adult fish captured in Esteros del Riachuelo, Corrientes, Argentina (27°25′S, 58°15′W). The reference interval and the mean were determined for each hematological parameter evaluated. Contrary to other species, the percent of heterophils was found to be high in relation to the percent of lymphocytes. Low lymphocyte counts occurred in *C. dimerus*, as compared to these found in other fishes. Compared to most teleosts, this species has similar mean values for PCV and Hb and slightly higher for RBC. Statistical analysis revealed that differences in hematological parameters between male and female fish were not significant.
© 2007 Published by Elsevier Ltd.

*Keywords:* Hematological analysis; Blood cells; Cichlidae; Teleostei

## 1. Introduction

Hematological indices are important parameters for the evaluation of fish physiological status. Their changes depend on the fish species, age, the cycle of sexual maturity and health condition (Blaxhall, 1972; Wedemeyer et al., 1983; Golovina and Trombicky, 1989; Zhiteneva et al., 1989; Bielek and Strauss, 1993; Golovina, 1996; Luskova, 1997; Vosylienė, 1999; Hrubec et al., 2001).

Hematological parameters are closely related to the response of the animal to the environment, an indication that the environment where fishes live could exert some influence on the hematological characteristics (Gabriel et al., 2004). These indices have been employed in effectively monitor-

ing the responses of fishes to the stressors and thus their health status under such adverse conditions. They can provide substantial diagnostic information once reference values are established under standardized conditions.

Evaluation of the hemogram involves the determination of the total erythrocyte count (RBC), total white blood cell count (WBC), hematocrit (PCV), hemoglobin concentration (Hb), erythrocyte indices (MCV, MCH, MCHC), white blood cell differential count and the evaluation of stained peripheral blood films (Campbell, 2004). Thrombocytes have been described as the most abundant blood cells after erythrocytes.

The South American cichlid fish *Cichlasoma dimerus*, a perciform teleost, is common in quiet shallow waters of the Paraguay and most of the Paraná river basins (Kullander, 1983). This freshwater species adapts easily to captivity and shows notable reproductive features such as a high spawn-

* Corresponding author. Tel.: +54 11 4576 3348; fax: +54 11 4576 3384.
*E-mail address:* grarey@bg.fcen.uba.ar (Rey Vázquez, G.).

ing frequency and acceptable survival rates, providing an appropriate model for laboratory studies.

The purpose of this study was to obtain a basic knowledge of the hematology of *C. dimerus*. In this paper, morphological features of the blood cells are described at the light and electron microscopic level. Assessment of hematological parameters in *C. dimerus* might provide some useful information for other researchers that could be used as a biomarker associated with stressors agents or as an available tool to diagnose and monitor disease in this species, which is representative of the ichthyich fauna in the La Plata River Basin.

## 2. Materials and methods

Adult specimens of *C. dimerus* used in this study were captured in Esteros del Riachuelo, Corrientes, Argentina (27°25′S, 58°15′W). Thirty fish were weighed and measured (12 females: 27.14 ± 8.95 g and 10.5 ± 1.17 cm, TL; 18 males: 50.15 ± 11.5 g and 12.78 ± 0.98 cm, TL) and kept in 70 l aquaria at 26.5 ± 1 °C, pH 7.3, with 12:12 h photoperiod and an average density of 6.4 g/l. Laboratory aquaria were well aerated and provided with external filtration and a layer of gravel on the bottom. Fish were normally fed once a day with pelleted commercial food (TetraCichlid food sticks). They were allowed to acclimate to captivity conditions for a month prior to taking the blood samples.

Careful netting and handling was implemented to minimize stress. The specimens were anesthetized with Fish Calmer (active ingredients: acetone, dimethylketone, alpha methyl quinoline, Jungle Laboratories, Cibolo, TX, USA), and the peripheral blood was collected by puncture of the caudal vein with a heparin-coated 25 gauge × 0.5 in. needle, attached to a 1 ml syringe. After sampling, fish were placed in separate tanks of freshwater for recovery.

Right after each extraction, blood samples were processed for microscopy as follows:

For light microscopy, a blood smears form every fish was fixed in absolute methanol for 3 min at room temperature or formalin vapour at 37 °C for 1 h, and stained with 10% Giemsa in PBS, hematoxylin–eosin or PAS (Martoja and Martoja, 1970; Pearse, 1980). The slides were subsequently examined and photographed under a Nikon Microphot FX.

For transmission electron microscopy (TEM), pellets of whole blood cells from five fish were obtained by centrifugation (400 g, 10 min, 4 °C) and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h. The blood was then rinsed in 0.1 M phosphate buffer and post-fixed in 1% osmium tetraoxide in 0.1 M phosphate buffer (pH 7.4) for 2 h. Subsequently, it was rinsed in distilled water, dehydrated in a graded alcohol series and acetone, and embedded in Spurr resin. Ultrathin sections were made with a Sorvall MT2-B ultramicrotome, stained with aqueous uranyl acetate and lead citrate, and examined afterwards under a Zeiss EM 109

T. Semithin sections, stained with toluidine blue, were used both for orientation and cell analysis.

Within the first 2 h after each extraction, the blood samples were processed for RBC, WBC and PCV as follows: RBC (Kaplow, 1955) and WBC + thrombocytes (Natt and Herrick, 1952) were determined using a Neubauer hemocytometer. Differential white cell and thrombocyte counts were done on blood films stained with Giemsa. For every 1200 erythrocytes counted at random, the number of thrombocytes and the different types of leucocytes was determined on each blood smear and a mean relative percent calculated. The absolute value was then obtained, multiplying this percent value by the WBC + thrombocytes from the hemocytometer. Thrombocyte numbers were subtracted from the WBC + thrombocytes count to obtain a total WBC. Replicate counts were made for each blood sample.

Hematocrit value was determined by the standard microhematocrit method, and expressed in percentage. Duplicate blood samples were loaded into standard heparinized capillary tubes, spun in a microhematocrit centrifuge at 12,000 rpm for 5 min and measured on a microcapillary reader.

Later Hb in erythrocytes was determined using the cyanmethaemoglobin method (hemogloWiener reactive, Wiener Lab.). Prior to reading the absorbance, hemoglobin test samples were centrifuged to remove dispersed nuclear material.

The following indices: mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) was calculated according to Seiverd (1964).

Size reference intervals were determined recording the smallest and greatest size measured for each cell type.

Differences in hematological parameters between male and females fish were statistically analyzed by Student's *t*-test.

## 3. Results

### 3.1. Cell morphology

Erythrocytes, thrombocytes and four types of leucocytes: lymphocytes, monocytes, heterophils and eosinophils were distinguished and characterized by light and transmission electron microscopy (Figs. 1 and 2; Table 1).

Erythrocytes are biconvex cells with an eosinophilic homogeneous cytoplasm and a central hump corresponding to the position of their oval nucleus. This centrally located nucleus contains condensed chromatin. Erythrocytes are lilac on preparations stained with Giemsa, blue with toluidine blue, while they are negative to PAS (Fig. 1A and F). Ultrastructurally, their nuclei are strongly heterochromatinic, while the cytoplasm is highly electron-dense and contains few organelles, only round vesicles being observed (Fig. 2A).

Thrombocytes present different morphologies in blood smears, from fusiform to spindle or oval-shaped, with an
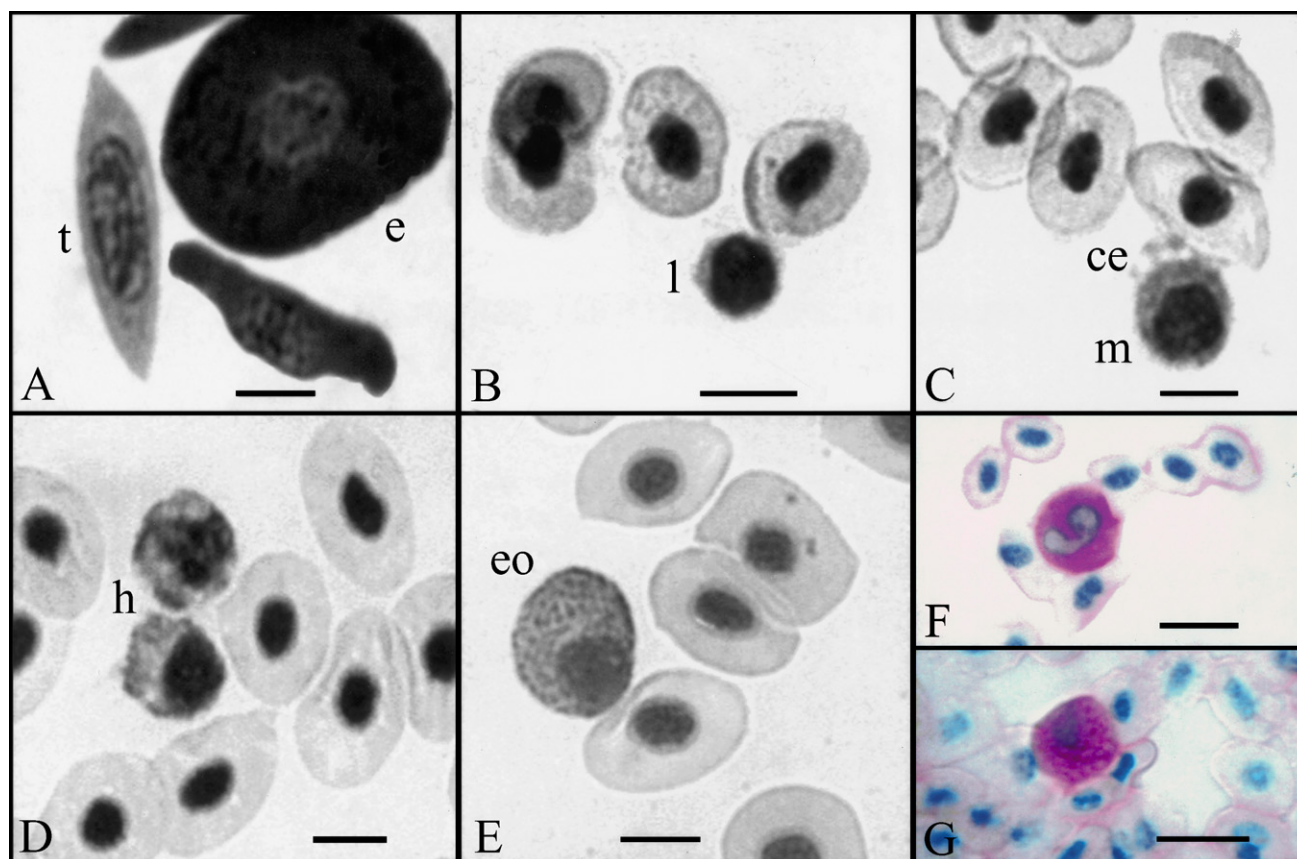
Fig. 1. Light microscopic micrographs of peripheral blood. (A) e, erythrocyte; t, thrombocyte. Toluidine blue. Scale bar = 2 μm. (B–E) Giemsa. (B) l, lymphocyte. Scale bar = 5 μm. (C) m, monocyte. Scale bar = 5 μm. (D) h, heterophilic granulocyte. Scale bar = 4 μm. (E) eo, eosinophilic granulocyte. Scale bar = 5 μm. (F–G) PAS. (F) Heterophilic granulocyte. Scale bar = 6 μm. (G) Eosinophilic granulocyte. Scale bar = 8 μm. ce, cytoplasmatic extensions.

oval and centrally located nucleus. They appear as single cells but usually occur in clusters. Giemsa failed to stain the cytoplasm of thrombocytes, but in the blood smears stained with toluidine blue, the cytoplasm appears with a light blue coloration (Fig. 1A). Under TEM, the nucleus exhibits prominent heterochromatin blocks which transverse it. Indentations of the plasma membrane are often observed. Cytoplasmic organelles are scarce. Only a large granule surrounded by membrane is observed next to the nucleus. A clear space is observed between the granule content and the bounding membrane (Fig. 2B).

Lymphocytes are small round cells. This basophilic cell type possesses a large and spherical centrally located nucleus that occupies most of the cell, and is surrounded by a thin rim

of light blue peripheral cytoplasm after Giemsa staining. The nucleus is rich in heterochromatin and appears violet in preparations stained with Giemsa (Fig. 1B). Ultrastructurally, the lymphocyte exhibits fingerlike cell processes. Its large central nucleus contains dense patches of heterochromatin and one nucleolus. The thin rim of cytoplasm contains numerous free ribosomes, mitochondria, rough endoplasmic reticulum and Golgi complex. The centriole is observed as well (Fig. 2C).

Monocytes are round to oval cells, characterized by a prominent, eccentric, kidney-shaped to bilobed nucleus. These cells exhibit a blue-purple nucleus and an agranular gray-blue cytoplasm after Giemsa staining. The peripheral region may be irregularly outlined in smears due to the formation of cytoplasmic extensions, characteristic of

Table 1
Light microscopic characteristics of *Cichlasoma dimerus* blood cells (Giemsa, 1000×)

| Cell | Cell size (μm) | Cell shape | Cytoplasm | Nuclear shape |
|---|---|---|---|---|
| Erythrocyte | 9.4–10 × 6.2–7.3 | Biconvex | Pink | Oval |
| Thrombocyte | 8.0–8.9 × 2.1–2.7 | Fusiform, spindle or oval | Translucent halo | Oval |
| Lymphocyte | 3.4–4.7 | Round | Thin pale blue rin | Spherical, conspicuos |
| Monocyte | 4.0–7.1 | Round | Gray-blue, homogeneous | Eccentric, kidney shape to bilobed |
| Heterophil | 5.0–9.5 | Round | Light purple, fine granules | Bilobed, eccentric |
| Eosinophil | 4.8–9.5 | Round | Large acidophilic granules | Round or bilobed, eccentric |
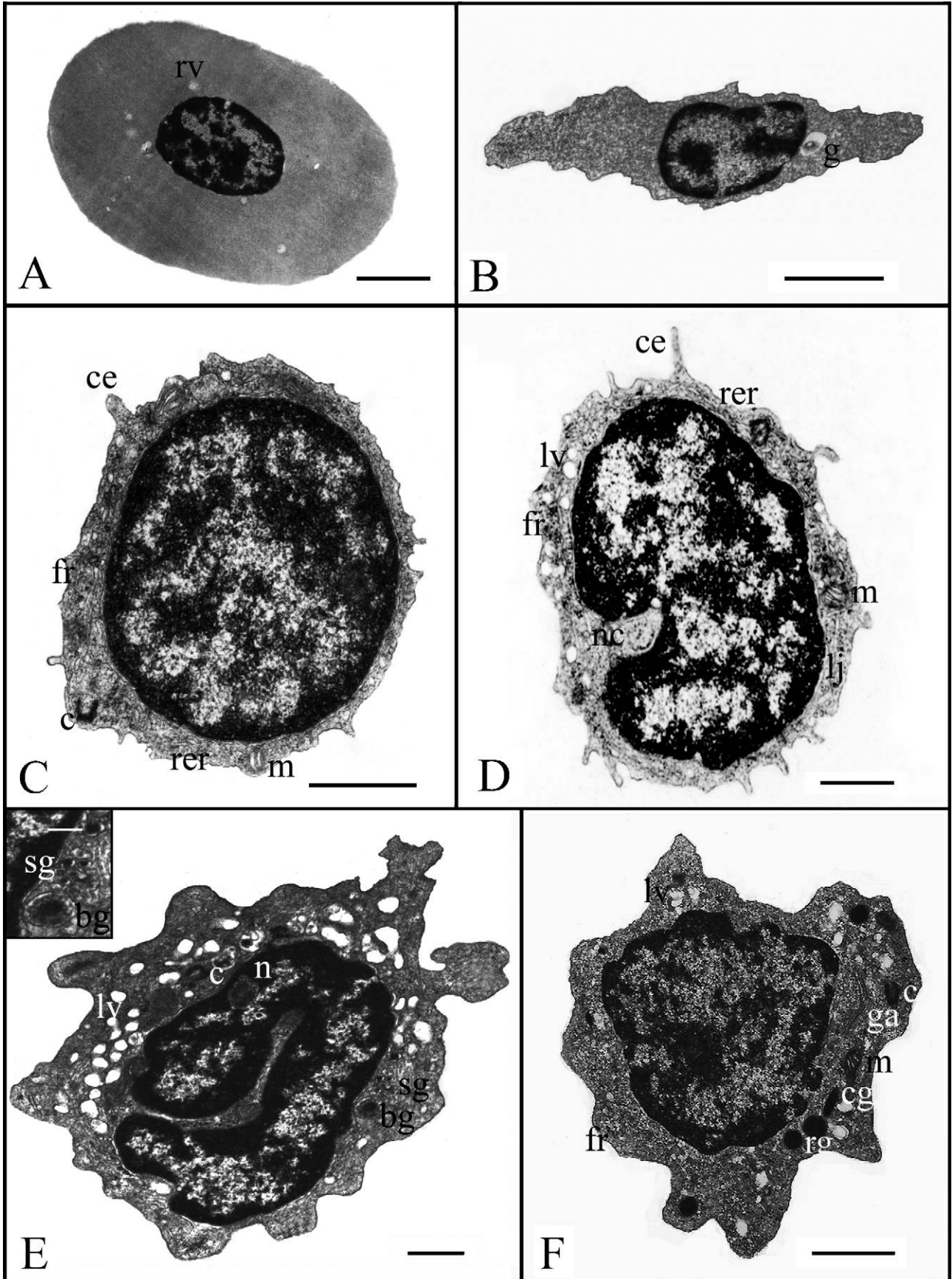
Table 2
Hematological parameters in *C. dimerus*

| Analyte | Mean ± S.D. | Reference interval | Sample size (*n*) |
| --- | --- | --- | --- |
| RBC ($\times 10^6\ \mu l^{-1}$) | 3.08 ± 0.97 | 1.68–4.27 | 30 |
| WBC ($\times 10^3\ \mu l^{-1}$) | 12.18 ± 3.84 | 6.64–18.59 | 30 |
| PCV (%) | 31.33 ± 4.97 | 22.5–39.12 | 30 |
| Hemoglobin (g/dl) | 6.82 ± 1.04 | 5.23–8.33 | 18 |
| MCV (fl) | 110.27 ± 38.16 | 70.14–198 | 30 |
| MCH (pg) | 24.48 ± 8.28 | 14.51–40.59 | 18 |
| MCHC (g/dl) | 22.32 ± 3.85 | 17.43–30.31 | 18 |
| Lymphocytes ($\times 10^3\ \mu l^{-1}$) | 4.68 ± 1.47 | 2.55–7.14 | 30 |
| Monocytes ($\times 10^3\ \mu l^{-1}$) | 2.19 ± 0.69 | 1.19–3.34 | 30 |
| Heterophils ($\times 10^3\ \mu l^{-1}$) | 3.43 ± 1.08 | 1.87–5.24 | 30 |
| Eosinophils ($\times 10^3\ \mu l^{-1}$) | 1.87 ± 0.59 | 1.02–2.85 | 30 |
| Thrombocytes ($\times 10^3\ \mu l^{-1}$) | 31.55 ± 9.95 | 17.2–48.15 | 30 |

actively phagocytic cells (Fig. 1C). Under TEM, the cytoplasm contains mitochondria, free ribosomes, numerous light vesicles, rough endoplasmic reticulum, Golgi complex, Golgi derived vesicles/granules. Centrioles are usually located in the nuclear cleft. A light juxtanuclear area is observed. Monocytes also contain heterophagosomes, attesting to their phagocytic activity (Fig. 2D).

### 3.2. Granulocytes

Heterophils are large and basophilic cells, commonly irregular in outline due the presence of short processes. The nucleus is horseshoe-shaped and bilobed (Fig. 1D). PAS staining demonstrates the presence of glycogen in the cytoplasm of this cell type (Fig. 1F). Ultrastructurally, the cytoplasm contains round to elongated granules, as well as numerous light vesicles, some of which exhibit a content inside. Golgi apparatus is occasionally observed. Rough endoplasmatic reticulum is less prominent than in other granulocytes. Two different populations of granules, with distinctive ultrastructural aspect, are distinguished. Some of them are small, and oval to round, containing homogeneous electron-dense material, while the others are big and round, and show a heterogeneous electron-dense core. In both type of granules, the content is surrounded by a light halo. The nucleus presents large peripheral heterochromatin blocks and a prominent nucleolus (Fig. 2E).

Eosinophils are big round, frequently irregularly outlined cells. They have an eccentric, round or sometimes bilobed nucleus. The cytoplasm is full of acidophilic granules that occasionally obscure the nucleus (Fig. 1E). Like heterophils, eosinophils are PAS positive (Fig. 1G). Ultrastructurally, their nuclei posses peripheral clumps of heterochromatin and a centrally located nucleolus. A juxtanuclear Golgi apparatus, light vesicles and free ribosomes are present in the cytoplasm.

Two types of granules are observed: round and cylindrical. Both of them show a homogeneous, electron-dense content surrounded by a light peripheral halo (Fig. 2F).

### 3.3. Hematological analysis

Results of hematological analysis are shown in Table 2, which includes the reference interval and the mean for each of the different parameters evaluated. A low count of lymphocytes is observed. The percent of heterophils is found to be high in relation to the percent of lymphocytes. Statistically analysis (*t*-test, $p < 0.05$) reveals that hematological parameters are not significantly different between male and female fish (data not shown).

## 4. Discussion and conclusions

There is growing interest in the study of hematological parameters and structural features of fish blood cells regarded as important for aquaculture purposes. A combination of quantitative and morphological methods is needed if the classification of fish blood cells is to advance from its present state.

Erythrocytes are the dominant cell type in the blood of the vast majority of fish species. It is widely accepted that fishes, like most other vertebrates, have a common leucocyte pattern consisting of granulocytes, monocytes, lymphocytes and thrombocytes.

In the present study, *C. dimerus* blood cells were characterized microscopically and hematological indices were analyzed. The mature erythrocytes of *C. dimerus* show an average size and ultrastructural features similar to those described for mature erythrocytes of other fish species and, like in all the species examined so far, they are the predomi-

Fig. 2. Electron microscopic micrographs of peripheral blood. (A) Erythrocyte. Scale bar = 2 μm. (B) Thrombocyte. Scale bar = 2 μm. (C) Lymphocyte. Scale bar = 1 μm. (D) Monocyte. Scale bar = 1 μm. (E) Heterophilic granulocyte. Scale bar = 1 μm. Inset: Detail of granules. Scale bar = 0.12 μm. (F) Eosinophilic granulocyte. Scale bar = 2 μm. bg, big granule; c, centriole; ce, cytoplasmatic extensions; cg, cylindrical granules; fr, free ribosomes; g, granule; ga, Golgi apparatus; lj, light juxtanuclear area; lv, light vesicles; m, mitochondria; n, nucleolus; nc, nuclear cleft; rg, rounded granules; rer, rough endoplasmatic reticulum; rv, round vesicles; sg, small granule.

Table 3
Cell size in microns (mean ± S.D. or reference interval) in some teleost fishes

| Species | Erythrocyte | Thrombocyte | Lymphocyte | Monocyte | Heterophil | Eosinophil | References |
|---|---|---|---|---|---|---|---|
| *Cyprynus carpio* | 10.2 × 13.4 | 4.6 × 7.7 | 6.6–11.8 | 10.0–16 | 10.0–15 | 13.8 | Groff and Zinkl (1999) |
| *Carassius auratus* | 7–9 × 12–14 | 4.7–5.6 | 7.4–8.4 | 7.0–17 | 10.2–12.1 | 7.4–8.4 | Groff and Zinkl (1999) |
| *Dicentrarchus labrax* | 10.47 ± 2.7 × 2.59 ± 0.62 | | 3.88 ± 0.87 | 6.02 ± 0.98 | 6.38 ± 1.04 | 5.39 ± 0.77 | Esteban et al. (2000) |
| *Oreochomis hybrid* | 12.9 × 7 | 4.7–5.5 | 4.6–5 × 5.7–6.4 | 9.4–10.7 | 6.38 ± 1.04 | 5.77–6.7 | Hrubec et al. (2000) |
| *Oryzias latipes* | | 7.0–10 | 6.0–9.0 | 8.0–23 | 7.0–20 | | Nakamura and Shimozawa (1984) |
| *C. dimerus* | 9.4–10 × 6.2–7.3 | 8.0–9 × 2.1–2.7 | 3.4–4.7 | 4.0–7.1 | 5–9.5 | 4.8–9.5 | Present study |

nant cell type found in the blood (Tables 3 and 5) (Watson et al., 1963; Hartman and Lessler, 1964; Conroy, 1972; Blaxhall and Daisley, 1973; Javaid and Akhtar, 1977; Nakamura and Shimozawa, 1984; Rowley et al., 1988; Groff and Zinkl, 1999; Hrubec et al., 2000; Esteban et al., 2000; Ueda et al., 2001).

Thrombocytes are the most abundant blood cells after erythrocytes, representing more than 50% of circulating leucocytes (Ueda et al., 1997). However, some authors do not include thrombocytes within leucocytes (Hrubec et al., 2000, 2001; Örün and Erdemli, 2002; Ranzani-Paiva et al., 2003). When smearing is carried out too slowly, or the blood is allowed to partially clot before smearing, then the thrombocytes are often seen in aggregates displaying a ragged appearance. When the blood is collected in a suitable anticoagulant such as sodium citrate or heparin, however, the thrombocytes appear oval or spindle shaped, and this is presumably their normal *in vivo* appearance (Rowley et al., 1988). The clusters of thrombocytes observed in some of the slides used for counting indicate that the blood is partially clotting, despite the use of an anticoagulant. As a result, cell counts might be not as accurate as expected, since blood cells are not evenly distributed. In teleost thrombocytes, only a single population of granules has been reported under TEM (Cannon et al., 1980a) and these have a clear space between the granule content and the bounding membrane, which is characteristic of lysosomes (Daems et al., 1972). The degree of granulation and vacuolation is variable and this has led some workers to suggest the existence of sub-populations (Zapata and Carrato, 1980; Pica et al., 1983). According to Rowley et al. (1988), this variation probably represents either different maturation stages of one cell type or some functional heterogeneity within the thrombocytes. In *C. dimerus*, one large cytoplasmatic granule, like that described in *Ictalurus punctatus* by Cannon et al. (1980a), is observed within each thrombocyte. Other prominent ultrastructural features of thrombocytes are the indentations of the cytoplasmatic membrane. By light microscopy, *C. dimerus* thrombocytes are recognized easily with respect to lymphocytes according to morphological features and size, as summarized in Table 1. In contrast, other authors failed to distinguish thrombocytes from lymphocytes according to size and morphology (see Rowley et al., 1988; Esteban et al., 2000). Table 3 shows the size of blood cells in different teleost species. Comparisons

reveal that *C. dimerus* thrombocytes are of similar size to those of *Oryzias latipes*.

Lymphocytes are usually the most common leucocyte type present in the blood of some fish, accounting for as much as 85% of the total leucocyte population, excluding thrombocytes (Groff and Zinkl, 1999). Several authors have demonstrated a special interest in the leucocytes of teleost fishes with regard to their morphology and absolute values. Their investigations revealed a great diversity of morphological aspects in some types of leucocytes (Srivastava, 1968; Blaxhall and Daisley, 1973; Ezzat et al., 1973; Ellis, 1976; Ferguson, 1976; Imagawa et al., 1989; Ueda et al., 1997; Veiga, 1999). In addition, the same type of leucocyte has been described with different names by different authors. Therefore the nomenclature concerning teleost leucocytes is confusing (Ueda et al., 2001). *C. dimerus* lymphocytes are similar in size (Table 3) and morphology to those of *Dicentrarchus labrax* (Esteban et al., 2000). Under TEM, they show fingerlike cell processes (cytoplasmatic extensions) (Fig. 2C). In *C. dimerus*, lymphocytes are less abundant than in other species (Table 5).

The nomenclature used to describe monocytes in fishes is variable. Monocytes have been termed hemoblasts and macrophages (Barber et al., 1981), while other authors have been unable to find monocytes (Blaxhall and Daisley, 1973). There are relatively few morphological studies on fish monocytes, limited to light microscopy (Cannon et al., 1980b; Page and Rowley, 1983; Ueda et al., 2001; Valenzuela et al., 2003) and electronic microscopy (Nakamura and Shimozawa, 1984; Esteban et al., 2000; Hrubec et al., 2000). *C. dimerus* monocytes show ultrastructural features similar to those of *O. latipes* (Nakamura and Shimozawa, 1984) and *D. labrax* (Esteban et al., 2000). Compared to the last species, they are of similar size as well (Table 3).

In fishes, granulocytes are of three types: heterophils and eosinophils are the most common, while basophils are much rare (Table 4). Most authors recognize heterophils as the most frequent type of granulocyte. The occurence of eosinophils, and in particular basophils, is often questioned (Ellis, 1977; Rowley et al., 1988). In the blood of *Oreochromis niloticus*, Ueda et al. (2001) identified all three types of granulocytes, however Rodrigues Bittencourt et al. (2003) failed to find basophils and eosinophils in this tilapia under semi-intensive culture conditions (Table 4). In *C. dimerus*, we report the

Table 4
Granulocyte heterogeneity in some Perciformes

| Species | Heterophilic granulocyte | Eosinophilic granulocyte | Basophilic granulocyte | References |
|---|---|---|---|---|
| *Blennius folis* | + (21.8) | − | − | Mainwaring in Rowley et al. (1988) |
| *Morone saxatilis* | + | + | − | Bodammer (1986) |
| *D. labrax* | + | + | − | Esteban et al. (2000) |
| *O. hybrid* | + | + | − | Hrubec et al. (2000) |
| *Oreochromis niloticus* | + | + | + | Ueda et al. (2001) |
| *O. niloticus* | + | − | − | Rodrigues Bittencourt et al. (2003) |
| *C. dimerus* | + | + | − | Present study |

Values in parentheses are the % of the total leucocyte count.

presence of heterophilic and eosinophilic granulocytes, while basophilic granulocytes have not been found (Table 4).

The cytochemical study of *O. niloticus* blood cells demonstrated the presence of glycogen in the cytoplasm of heterophils, thrombocytes, lymphocytes and monocytes (Ueda et al., 2001). In *C. dimerus*, only heterophils and eosinophils were found to be PAS positive (Fig. 1F and G).

Ultrastructurally, the heterophils of *I. punctatus* have been characterized by having a cytoplasm filled with rod-shaped granules, which in the mature form have a crystalline or striated appearance in the center (Cannon et al., 1980a). Similar substructures of these granules have been found in the heterophils of *B. pholis* (Rowley et al., 1988), *P. platessa* (Ferguson, 1976) and *Morone saxatilis* (Bodammer, 1986). In these species, heterophils present only a single population of granules in the cytoplasm.

Garavini and Martelli (1981) stated that two types the granules are formed in immature and mature goldfish heterophils. In *C. carpio*, the substructure of heterophil granules is similar to that of the eosinophil granules of *C. auratus* (Page and Rowley, 1983). In *C. dimerus*, heterophils are the most commonly encountered type of granulocyte and they have similar ultrastructural characteristics to those of *D. labrax* (Esteban et al., 2000). However, while three types of granules were described in the heterophils of *D. labrax*, *C. dimerus* heterophils present two types (Fig. 2E).

*C. dimerus* eosinophils are big cells, being exceeded in size only by those of the common carp, *C. carpio* (Groff and Zinkl, 1999) (Table 3). They share similar characteristics with *D. labrax* eosinophils (Esteban et al., 2000) and present two types of granules (Fig. 2F).

The knowledge of the hematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Kori-Siakpere et al., 2005). Previous studies on fish hematology have revealed that interpretation of blood parameters is quite difficult since variations in the blood are caused by internal and external factors. It is well known that blood sampling, laboratory techniques, seasonal variations, size, genetic properties, sex, population density, lack of food supply, environmental stress and transportation could affect hematological data (Ezzat et al., 1973; Denton and Yousef, 1975; Fourtie and Hatting, 1976; Van Vuren and Hattingh, 1978; Hardig and Hoglung, 1984; Wilhem et al., 1992; Örün

and Erdemli, 2002; Arnold, 2005; Kori-Siakpere et al., 2005). For this reason, researchers must be careful when establishing reference intervals and making comparisons. Often, a direct comparison is not possible because of the different experimental conditions.

The study of normal hematological characteristics in *Tilapia zilli* revealed predominance of lymphocytes and heterophils, followed by monocytes and eosinophils (Ezzat et al., 1973). On the other hand, a high percentage of heterophils, monocytes and lymphocytes was reported in *Orechromis aureus* (Silveira and Rigores, 1989), while in *O. niloticus*, also in normal conditions, heterophils and lymphocytes predominated, followed by monocytes, rarely basophils and eosinophils (Ueda et al., 1997). The most abundant types of leucocytes found in the peripheral blood of *C. dimerus* were the lymphocytes and heterophils (Table 2), as demonstrated in *Pimelodus maculatus* (Ribeiro, 1978), *Synbranchus marmoratus* (Nakamoto et al., 1991), *Mugil platanus* (Ranzani-Paiva, 1995) and *Oncorhynchus mykiss* (Ranzani-Paiva et al., 1998). A similar pattern was reported in *C. carpio* and *C. auratus* (Groff and Zinkl, 1999), *Oreochromis* hybrid (Hrubec et al., 2000), *Morone* hybrid (Hrubec et al., 2001), *Capoetta trutta* (Örün and Erdemli, 2002) and *Salminus maxillosus* (Ranzani-Paiva et al., 2003), although the relative ratio changed according to the species (Table 5).

Under the conditions employed in this study, the lymphocyte count resulted in the same order of magnitude as that of the heterophil count. Regarding heterophils, the numbers obtained in this research agree with those reported in the goldfish *C. auratus* (Groff and Zinkl, 1999). Low lymphocyte count occurred in *C. dimerus* compared to that found in other species (Table 5). According to Campbell (2004), it is probable that the low density conditions and optimal water quality resulted in a healthy situation that inhibited the lymphocytosis produced during wound healing, inflammatory disease, parasitic infections and viral diseases. Even though we have not had difficulties to distinguish lymphocytes, these results required us to discard probable technical mistakes in the method used for identifying them.

Collated with other species, *C. dimerus*, presents similar mean values for PCV and Hb and slightly higher for RBC, although this RBC value resulted exactly the same than that reported in *Morone* hybrid (Table 5). No statistically signif-

Table 5
Hematology (reference interval or mean ± S.D.) in teleost fishes

| Analyte | Species | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *C. carpio* (mean ± S.D. or interval) | *C. auratus* (mean ± S.D.) | *O. hybrid* (reference interval) | *Morone hybrid* (reference interval) | *Capoeta trutta*[a] (mean ± S.D.) | *Salminus maxillosus* (mean ± S.D.) | *Parachenna obscura* (mean ± S.D.) | *C. dimerus* (mean ± S.D.) |
| HCT (%) | 33.4 ± 1.51 | 22.3 ± 1.04 | 27–37 | 29–36 | 26.05 ± 2.38 | – | 19 ± 3.8 | 31.33 ± 4.97 |
| Hemoglobin (g/dl) | 8.2 ± 0.36 | 6.7 ± 0.25 | 7.0–9.8 | 7.3–9.4 | 7.9 ± 0.24 | | 5.7 ± 1.24 | 6.82 ± 1.04 |
| MCV (fl) | 202 ± 5.5 | 137 ± 2.6 | 115–183 | 78–102 | 149.71 ± 2.28 ($\mu$l$^3$) | – | 132.84 ± 5.56 ($\mu$l$^3$) | 110.27 ± 38.16 |
| MCH (pg) | 49.1 | 42 ± 1.4 | 28.3–42.3 | 19–25 | 45.4 ± 1.8 | – | 39.89 ± 17.36 | 24.48 ± 8.28 |
| MCHC (g/dl) | | | 22–29 | 22–27 | 30.32 ± 0.8 (%) | | 29.08 ± 5.9 (%) | 22.32 ± 3.85 |
| RBC ($\times 10^6$ $\mu$l$^{-1}$) | 1.67 ± 0.08 | 1.61 ± 0.81 | 1.91–2.83 | 3.15–4.22 | 1.74 ± 0.12 | – | 1.67 ± 0.708 | 3.08 ± 0.97 |
| WBC ($\times 10^3$ $\mu$l$^{-1}$) | 37.8 ± 2.88[b] | 52.3 ± 4.88[b] | 21.5–154.7 | 12.1–13.1 | 17.65 ± 2.15 | | 19.07 ± 7.4 | 12.8 ± 3.84 |
| Lymphocytes ($\times 10^3$ $\mu$l$^{-1}$) | 32.26–35.15 | 26.7 ± 2.89 | 6.8–13.64 | 9.2–21.8 | 13.1 ± 0.01 | 53.1 ± 1.4 | – | 4.68 ± 1.47 |
| Heterophils ($\times 10^3$ $\mu$l$^{-1}$) | 1.13–3.78 | 2.3 ± 0.56 | 0.5–9.873 | 0.1–2.4 | 2.56 ± 0.12 | 33.3 ± 1.3 | – | 3.43 ± 1.08 |
| Monocytes ($\times 10^3$ $\mu$l$^{-1}$) | 0.19–0.76 | 0.2 ± 0.1 | 0.4–4.3 | 0.2–2.5 | 1.9 ± 0.07 | 5.8 ± 0.4 | – | 2.12 ± 0.69 |
| Eosinophlis ($\times 10^3$ $\mu$l$^{-1}$) | 0.19–0.38 | 0.1 ± 0.1 | 0.035–1.645 | 0–1.6 | 0.075 ± 0.005 | 2.2 ± 0.1 | – | 1.87 ± 0.59 |
| Thrombocytes ($\times 10^3$ $\mu$l$^{-1}$) | 12.6 ± 3.5 | 19.4 ± 3.9 | 25–85.2 | 19.5–74.5 | 0.691 ± 0.03 | – | – | 31.55 ± 9.95 |
| References | Groff and Zinkl (1999) | Groff and Zinkl (1999) | Hrubec et al. (2000) | Hrubec et al. (2001) | Örün and Erdemli (2002) | Ranzani-Paiva et al. (2003) | Kori-Siakpere et al. (2005) | Present study |

[a] Four years old adult specimens.

[b] WBC included thrombocytes.

icant variations were found in the level of these parameters between sexes.

The erythrocyte indexes MCV and MCH have a wide range of physiological variation. The MCV of *C. dimerus* is similar to that of *C. auratus* and *Morone* hybrid, while the MCH agrees with that of *Morone* hybrid, *Oreochromis* hybrid and *P. obscura* (Table 5). Arnold (2005) argued that in elasmobranches, manual RBC lacks the precision necessary for the accurate assessment of anemia or for calculating accurate MCV and MCH values. Blaxhall and Daisley (1973), working with teleosts, also concluded that manual RBC counts were error proved and suggested that PCV and Hb may be better parameters for the assessment of anemia in fish. Our experience indicates that even though PCV and Hb are better indices for the assessment of anemia in *C. dimerus*, manual RBC is an accurate enough method, since evident differences in the number of red blood cells were recorded in fish exposed to a chemical stress, as compared to fish from control treatments (unpublished data).

In summary, the results of our research provide a contribution to the knowledge of the characteristics of blood cells and hematological parameters of the cichlid fish, *C. dimerus*, under the normal conditions employed in this study. This investigation may be helpful as a tool to monitor the health status of this and other related fish species. The evaluation of hematological parameters will grant early detection of clinical pathology as well as the presence of disturbance in the environment.

## Acknowledgements

## References

Arnold, J.E., 2005. Hematology of the sandbar shark, *Carcharinus plumbeus*: standardization of complete blood count techniques for elasmobranches. Vet. Clin. Pathol. 34 (2), 115–123.

Barber, D.L., Westermann, J.E.M., White, M.G., 1981. The blood cells of the Antarctic icefish *Chaenocephalus aceratus* Lönnberg: light and electron microscopic observations. J. Fish Biol. 19, 11–28.

Bielek, E., Strauss, B., 1993. Ultrastructure of the granulocyte of the South American lungfish, *Lepidosiren paradoxa*: morphogenesis and comparison to other leucocytes. J. Morphol. 218, 29–41.

Blaxhall, P.C., 1972. The haematological assessment of the health of the freshwater fish. A review of selected literature. J. Fish Biol. 4, 593–604.

Blaxhall, P.C., Daisley, K.W., 1973. Routine haematological methods for use with fish blood. Fish Biol. 5, 771–781.

Bodammer, J.E., 1986. Ultrastructural observations on peritoneal exudate cells from striped bass. Vet. Immunol. Immunopathol. 12, 127–140.

Campbell, T.W., 2004. Hematology of lower vertebrates. In: Proceedings of the 55th Annual Meeting of the American College of Veterinary Pathologists (ACVPC) & 39th Annual Meeting of the American Society of Clinical Pathology (ASVCP). ACVP and ASVCP, USA.

Cannon, M.S., Mollenhauer, H.H., Eurell, T.E., Lewis, D.H., Cannon, A.M., Tompkins, C., 1980a. An ultrastructural study of the leucocytes of the channel catfish, *Ictalurus punctatus*. J. Morphol. 164, 1–23.

Cannon, M.S., Mollenhauer, H.H., Cannon, A.M., Eurell, T.E., Lewis, D.E., 1980b. Ultrastructural localization of peroxides activity in neutrophil leucocytes of *Ictalurus punctatus*. Can. J. Zool. 58, 1139–1143.

Conroy, D.A., 1972. Studies on the haematology of the Atlantic salmo (*Salmo salar*) L Symp. Zool. Soc. Lond. 30, 101–127.

Daems, W.Th., Wise, E., Brederoo, P., 1972. Electron microscopy of the vacuolar apparatus in lysosomes. In: Dingle, J.T. (Ed.), A Laboratory Handbook. North-Holland Publishers, Amsterdam, pp. 150–199.

Denton, J.E., Yousef, M.K., 1975. Seasonal changes in haematology of rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol. 51 (A), 151–153.

Ellis, A.E., 1976. Leucocytes and related cells in the plaice *Pleuronectes platessa*. J. Fish Biol. 8, 147–153.

Ellis, A.E., 1977. The leucocytes of fish: a review. J. Fish Biol. 11, 453–491.

Esteban, M.A., Muñoz, J., Meseguer, J., 2000. Blood cells of sea bass (*Dicentrarchus labrax* L.). Flow cytometric and microscopic studies. Anat. Rec. 258 (1), 80–89.

Ezzat, A.A., Shabana, M.B., Farghaly, A.M., 1973. Studies on the blood characteristics of *Tilapia zilli* (Gervais). I. Blood cells. J. Fish Biol. 6, 1–12.

Ferguson, H.W., 1976. The ultrastructure of the plaice (*Pleuronectes platessa*) leucocyte. J. Fish Biol. 8, 139–412.

Fourtie Jr., F., Hatting, J., 1976. A seasonal study of the haematology of carp (*Ciprinus carpio*) from a locality in the Transvaai. S. Afr. Zool. 11, 75–80.

Gabriel, U.U., Ezeri, G.N.O., Opabunmi, O.O., 2004. Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch, 1822). Afr. J. Biotechnol. 3, 463–467.

Garavini, C., Martelli, P., 1981. Alkaline phosphatase and peroxidase in goldfish (*Carassius auratus*) leucocytes. Basic Appl. Histochem. 25, 133–139.

Golovina, N.A., 1996. Morphofunctional characteristics of the blood of fish as objects of aquiculture. Doctoral Thesis. Moscow, p. 53.

Golovina, N.A., Trombicky, I.D., 1989. Haematology of Pond Fish. Kishinev, Shtiinca, p. 158.

Groff, J.M., Zinkl, J.G., 1999. Hematology and clinical chemistry of Cyprinid fish. Common carp and goldfish. Vet. Clin. N. Am. Exot. Anim. Pract. 2 (3), 741–746.

Hardig, J., Hoglung, L.B., 1984. Seasonal variation in blood components of reared Baltic Salmon, *Salmo salar*. J. Fish Biol. 24, 565–579.

Hartman, F.A., Lessler, M.A., 1964. Erythrocyte measurements in fishes, amphibian and reptiles. Biol. Bull. Woods Hole 126, 83–88.

Hrubec, T.C., Cardinale, J.L., Smith, S.A., 2000. Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* hybrid). Vet. Clin. Pathol. 29 (1), 7–12.

Hrubec, T.C., Smith, S.A., Robertson, J.L., 2001. Age related in haematology and chemistry values of hybrid striped bass chrysops *Morone saxatilis*. Vet. Clin. Pathol. 30 (1), 8–15.

Imagawa, T., Hashimoto, Y., Kitagawa, H., Kon, Y., Kudo, N., Sugimura, N., 1989. Morphology of the cell in carp (*Cyprinus carpio* L.). Jpn. J. Vet. Sci. 51, 1163–1172.

Javaid, M.Y., Akhtar, N., 1977. Haematology of fishes in Pakistan. II. Studies on fourteen species of teleosts. Biologia 23, 79–90.

Kaplow, L.S., 1955. A histochemical procedure for localizing and valuating leukocyte alkaline phosphatase activity in smears of blood and marrow. Blood 10, 1023.

Kori-Siakpere, O., Ake, J.E.G., Idoge, E., 2005. Haematological characteristics of the African snakehead, *Parachacnna obscura*. Afr. J. Biotechnol. 4 (6), 527–530.

Kullander, S.O., 1983. A Revision of the South American Cichlid Genus *Cichlasoma* (Teleostei: Cichlidae). Swedish Museum of Natural History, Stockholm, p. 296.

Luskova, V., 1997. Annual cycles and normal values of haematological parameters in fishes. Acta Sc. Nat. Brno. 31 (5), 70.

Martoja, R., Martoja, M., 1970. Técnicas de Histología Animal. Toray-Masson S.A., Barcelona, p. 350.

Nakamoto, W., Silva, A.J., Machado, P.E.A., Padovani, C.R., 1991. Glóbulos brancos e *Cyrilia gomesi* (hemoparasita) em *Synbrachus marmoratus* Bloch, 1795 (Pisces, Synbranchidae) da região de Birigui, SP. Rev. Bras. Biol. 51 (4), 755–761.

Nakamura, H., Shimozawa, A., 1984. Light and electron microscopic studies on the leucocytes of the medaka. Medaka 2, 15–22.

Natt, M.P., Herrick, C.A., 1952. A new blood diluent for counting erythrocytes and leucocytes of the chicken. Poult. Sci. 31, 735–738.

Örün, I., Erdemli, A.U., 2002. A study on blood parameters of *Capoeta trutta* (Heckel, 1843). J. Biol. Sci. 2 (8), 508–511.

Page, M., Rowley, A.F., 1983. A cytochemical, light and electron microscopical study of the leucocytes of the adult river lamprey, *Lampetra fluviatilis* (L. Gray). J. Fish Biol. 22, 503–517.

Pearse, A.G.E., 1980. Histochemistry Theoretical and Applied, vols. I and II. Churchill Livingstone Ed., London, p. 1055.

Pica, A., Grimaldi, M.C., Della Corte, F., 1983. The circulating blood cells of torpedoes (*Torpedo marmorata* Russo and *Torpedo ocellata* Rafinesque). Monit. Zool. 17, 353–374.

Ranzani-Paiva, M.J.T., 1995. Células sangüíneas e contagem diferencial dos leucócitos de tainha, *Mugil platanus* Günther, 1880 (Osteichthyes, Mugilidae) da região estuarino-lagunar de Cananéia, SP (Lat. 25°00′S–Long. 47°55′W). Bol. Inst. Pesca 22 (1), 23–40.

Ranzani-Paiva, M.J.T., Tabata, Y.A., Eiras, A.C., 1998. Hematologia comparada entre diplóides e triplóides de truta arco-íris, *Oncorhynchus mykiss* Walbaum (Pisces, Salmonidae). Rev. Bras. Zool. 5 (4), 1093–1102.

Ranzani-Paiva, M.J.T., Rodríguez, E.L., Veiga, M.L., Eiras, A.C., Campos, B.E.S., 2003. Differential leucokocyte counts in "dourado", *Salminus maxillosus* Valenciennes, 1840, from the Mogi-Guaçu River, Pirassununga, SP. Braz. J. Biol. 63 (3), 517–525.

Ribeiro, W.R., 1978. Contribuiçao ao estudo da haematologia de peixes. Morfología e histoquímica das células do sangue e dos tecidos haematopoéticos do mandi amarelo, *Pimelodus maculatus* Lacepede, 1803. Disertaçao (Doutorado), Faculdade de Medicina de Ribeirao Preto. Universidade de Sao Paulo, Ribeirao Preto, 119 f.

Rodrigues Bittencourt, N.L., Molinari, L.M., Scoaris, D.O., Bocchi Pedroso, R., Vatura Nakamura, C., Ueda-Nakamura, T., Filho, B.A.A., Dias Filho, B.P., 2003. Haematological and biochemical values for Nile tilapia *Oreochromis niloticus* cultured in semi-intensive system. Acta Sci. Biol. Sci. (Maringá) 25 (2), 385–389.

Rowley, A.F., Hunt, T.C., Page, M., Mainwaring, G., 1988. Fish. In: Rowley, A.F., Ratcliffe, N.A. (Eds.), Vertebrate Blood Cells. Cambridge University Press, Cambridge, pp. 19–127.

Seiverd, C.E., 1964. Haematology for Medical Technologists. Lea and Febiger, Philadelphia, p. 946.

Silveira, R., Rigores, C., 1989. Características hematológicas normales de *Oreochromis aureus* en cultivo. Rev. Latinoam. Acuic. Lima 39, 54–56.

Srivastava, A.K., 1968. Studies on the haematology of certain freshwater teleosts. Anat. Anzeiger 123, 520–533.

Ueda, I.K., Egami, M.I., Sasso, W.S., Matushima, E.R., 1997. Estudos hematológicos em *Oreochromis* (*Tilapia*) *niloticus*. (Linnaeus, 1758) (Cichlidae, Teleostei)—Part I. Braz. J. Vet. Res. Anim. Sci. 34 (5), 270–275.

Ueda, I.K., Egami, M.I., Sasso, W.S., Matushima, E.R., 2001. Cytochemical aspects of the peripheral blood cells of *Oreochromis* (Tilapia) *niloticus*. (Linnaeus, 1758) (Cichlidae, Teleostei)—Part II. Braz. J. Vet. Res. Anim. Sci. 38 (6), 273–277.

Valenzuela, A., Oyarzún, C., Silba, V., 2003. Blood cells of the *Schroederichtys chlensis* (Guichenot, 1848): the leukocytes (Elasmobranchii, Scyliorhinidae). Gayana 67 (1), 130–137.

Van Vuren, J.H.J., Hattingh, J., 1978. A seasonal sudy of haematology of wild freshwater fish. J. Fish Biol. 13, 305–313.

Veiga, M.L., 1999. Aspectos morfológicos das células sangüineas, citoquímicos e ultraestruturais de trombocitos, neutrófilos e eosinófilos de Dourado *Salminus maxillosus* (Valenciennes, 1840) (Pisces, Characidae). Dissertação (Mestrado) Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, 95 f.

Vosyliené, M.Z., 1999. The effect of heavy metals on haematological indices of fish. Acta Zool. Litvanica Hydrobiol. 9 (2), 76–82.

Watson, L.J., Shechmeister, I.L., Jackson, L.L., 1963. The haematology of the goldfish, *Carassius auratus*. Cytologia 28, 118–130.

Wedemeyer, G.A., Gould, R.W., Yasutake, W.T., 1983. Some potentials and limits of the leucocrit test as a fish health assessment method. J. Fish Biol. 23, 711–716.

Wilhem, D.F., Eble, G.J., Kassner, F.X., Dafré, A.L., Ohira, M., 1992. Comparative hematology in marine fish. Comp. Biochem. Physiol. 102, 311–321.

Zapata, A., Carrato, A., 1980. Ultrastructure of elamsmobranch and teleost thrombocytes. Acta Zool. (Stockholm) 61, 179–182.

Zhiteneva, L., Poltavceva, T.G., Rudnickaja, O.A., 1989. Atlas of normal and pathological cells in the blood of fish. Rostov-on-Don, p. 112.