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HEMATOLOGICAL DATA ON THE FISHES OF THE GENUS MUGIL LINNAEUS

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A great number of papers have been published on the study of the blood of marine and freshwater fishes. They approached not only the immunological and biochemical point of views, but the cytology and cytoformation aspects.

The present paper is a contribution to the knowledge of the hematology of mullets of the genus *Mugil* Linnaeus from the northeastern Brazil. Only the species *Mugil curema* Cuvier & Valenciennes and *Mugil incilis* Hancock were considered.

MATERIAL AND METHODS

Blood samples were drawn from 12 *Mugil curema* and 9 *Mugil incilis* captured through a beach net at Mucuripe Beach, localized in the basin of the port of Fortaleza (Ceará — Brazil), in the period from October 1967 to October 1968. The blood were collected from the gills vessels, using heparin as anticoagulant. Hemolysed or clotted samples were discarded.

Red blood cell count was done in a Neubauer chamber, using Hayem solution as a diluent fluid; hemoglobin was performed by Sahli method; the white blood cell count was determined by indirect method, counting in a stained slide, with oil immersion lens, the number of leukocytes in relation to 5,000 red cells; the thrombocytes were counted by Fonio's method (Janini, 1959). The leukocyte differential count was performed in 200 cells.

PAS reaction was done according McManus technique and Sudan Black staining, according the method recommended by Sheehan and Storey (Hayhoe *et al.*, 1964).

Fork length was determined in all specimens; sex identification and gonadal development were not considered.

We found one *Mugil curema* with hematological abnormalities. The data of this fish were studied apart in the present paper.

RESULTS

1 — *Mugil curema*

Red blood cells — the red blood cell count varied from 2,600,000 to 4,400,000 per cu.mm, with the mean value of 3,500,000 per cu.mm. No correlation was found between the red blood cell count and the fork length ($r = 0.09$).

Hemoglobin — the hemoglobin determination ranged from 7.0 to 12.8 g%, with the mean value of 10.4 g%. No correlation was found between the hemoglobin level and the fork length ($r = 0.23$).

White blood cell — the leukocyte count varied from 12,400 to 39,600 per cu.mm, with the mean value of 23,032 per cu.mm. The differential count performed in 12 fish was: neutrophils — 4.0%; lymphocytes — 88.0%; monocytes (macrophages according to Jakowska, 1956) — 8.0%. No correlation was found between the white blood cell count and the fork length ($r = 0.01$).

Thrombocytes — the thrombocyte level varied from 11,910 to 38,400 per cu.mm, with the mean value of 22,272 per cu.mm. No corre-

lation was found between the thrombocyte count and the fork length (r = 0.53) .

Fork length — the fork length ranged from 159 to 275 mm , with the mean value of 221 mm .

2 — *Mugil incilis*

Red blood cells — the red blood cell count varied from 3,500,000 to 5,000,000 per cu.mm , with the mean value of 4,300,000 per cu.mm . No correlation was found between the red blood cell count and the fork length (r = 0.29) .

Hemoglobin — the hemoglobin level ranged from 10.0 to 15.2 g% , with the mean value of 11.9 g% . No correlation was found between the hemoglobin value and the fork length (r = 0.05) .

White blood cells — the leukocyte count varied from 8,520 to 32,760 per cu.mm , with the mean value of 20,674 per cu.mm . The differential count performed in 9 specimens was: neuthrophils — 5.0% ; lymphocytes — 83.0% ; monocytes (macrophages according to Jakowska, 1956) — 12.0% . No correlation was found between the white blood cell count and the fork length (r = 0.27) .

Thrombocytes — the thrombocyte count ranged from 10,410 to 59,760 per cu.mm , with the mean value of 25,187 per cu.mm . No correlation was found between the thrombocyte count and the fork length (r = 0.01) .

Fork length — the fork length varied from 183 to 278 mm , with the mean value of 236 mm .

3 — Cytology and cytochemistry

Red blood cells — the red blood cells are elliptical with a central nucleus; round-shaped erythrocytes were also observed. Immature forms were seen in the periphery; these forms present a discrete polychromatophilia, and the chromatin is less compact. Red blood cells with nucleus in kariolysis and free fragments of nucleus were observed frequently. In *Mugil curema* the larger cell diameter ranged from 8.76 to 11.68 micra ; the larger nucleus diameter varied from 2.19 to 4.38 micra. In *Mugil incilis* the larger cell diameter ranged from 7.30 to 11.68 micra; the larger nucleus diameter ranged from 2.92 to 4.38 micra.

White blood cells — we observed in both species the same type of cells, with similar cytomorphology.

Lymphocytes — the lymphocytes are small, round with a large relation nucleus — cytoplasm (figure 1) . The chromatin is compact and no nucleoli were seen. The cytoplasm is scant, basofilic, without granules. The *Mugil curema* cell diameter ranged from 3.65 to 7.30 micra; and nucleus diameter from 2.92 to 5.11 micra . The *Mugil incilis* cell diameter ranged from 4.38 to 7.30 micra ; and nucleus diameter from 2.92 to 5.84 micra .

On PAS reaction, the majority of the cells are negative or showed tinge positivity of the cytoplasm; some lymphocytes have fine granules arranged as a crown around the periphery of the cell. The Sudan Black stain was negative.

Monocytes — (macrophages according to Jakowska, 1956) — the monocytes are large cells; in both species the cell diameter varied from 8.76 to 14.60 micra and the nucleus diameter from 5.11 to 9.49 micra (figure 2) . The nucleus may be central or excentric, round and sometimes may have indentations; a fine reticulated chromatin is seen, without nucleoli. The cytoplasm is abundant, slightly basophilic, with azurophil granules.

The PAS reaction and Sudan Black stain showed a fine granular positivity.

Neutrophilic granulocytes — the neutrophilic granulocytes are cells of medium size, round, with abundant cytoplasm and specific granules. The nuclei are round or band forms, with coarse chromatin; they have less affinity to the dies than the nuclei of lymphocytes (figure 3) . The observed diameters were — in *Mugil curema* the larger cell diameter ranged from 7.30 to 14.60 micra and the larger nucleus diameter from 4.38 to 8.76 micra; in *Mugil incilis* the larger cell diameter ranged from 9.49 to 14.60 micra and the larger nucleus diameter from 5.11 to 9.49 micra.

All the neutrophilic granulocytes present a strong homogeneous PAS positivity; on Sudan Black stain show granular positivity.

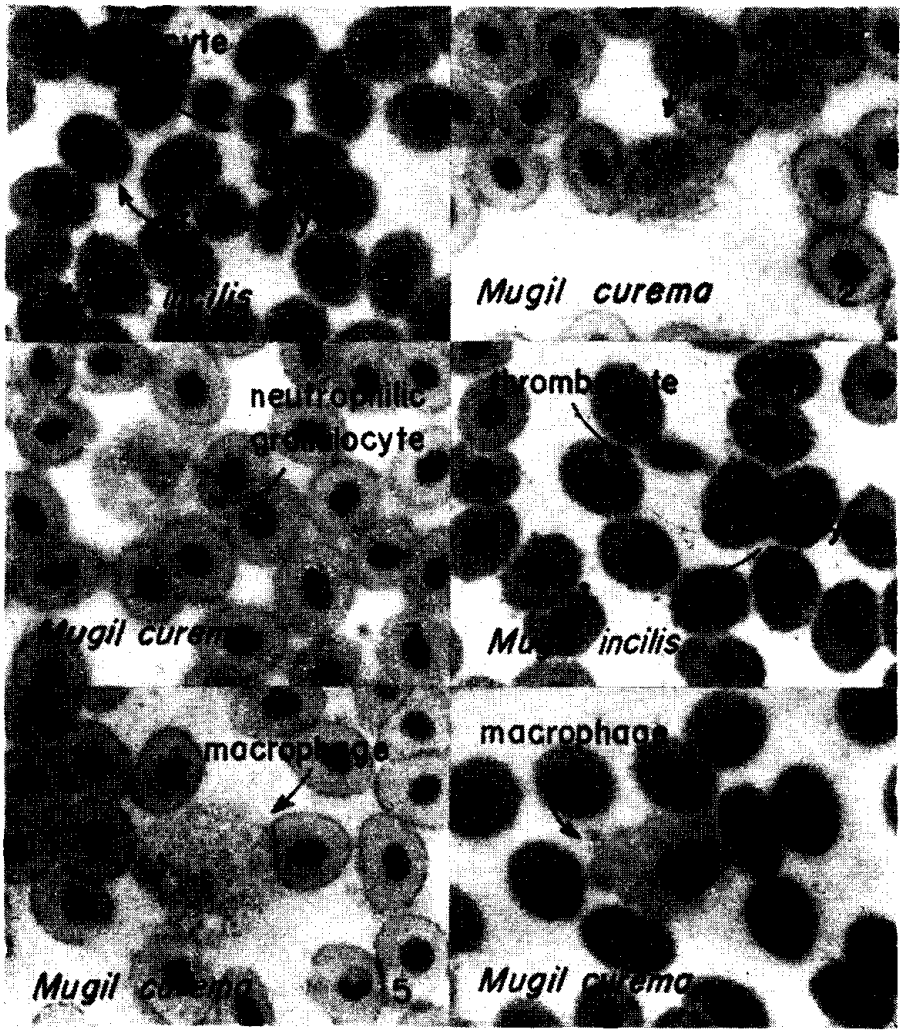
Thrombocytes — the thrombocytes are elliptical, but oval and round forms may be seen (figure 4) . The chromatin is compact; the cytoplasm is scant, acidophil, without granules.

PAS reaction shows tinge positivity and sometimes fine granules.

In *Mugil curema,* the larger cell diameter of thrombocytes varied from 5.84 to 11.68 micra and the smaller from 2.92 to 5.84 micra; the larger nucleus diameter ranged from 4.38 to 6.57 micra and the smaller from 2.19 to 2.92 micra. In *Mugil incilis,* the larger cell diameter of thrombocytes varied from 6.57 to 11.68 micra and the smaller from 2.92 to 5.84 micra; the larger nucleus diameter ranged from 4.38 to 7.30 micra and the smaller from 2.19 to 3.65 micra.

4 — Hematological abnormalities

In one specimen of *Mugil curema,* with the fork length of 210 mm , we found qualitative and quantitative leukocyte abnormalities. The following results were found: red



Figures 1 - 6 — Blood cell types of the mullets *Mugil curema* Cuvier & Valencienes and *Mugil incilis* Hancock, from northeastern Brazil.

blood cells — 4,350,000 per cu.mm ; hemoglobin — 12.2 g% ; white blood cells — 132,240 per cu.mm ; lymphocytes — 77.0% (101,825 per cu.mm) , neutrophilic granulocytes — 1.0% (1,322 per cu.mm) , monocytes — 22.0% (42,322 per cu.mm) ; thrombocytes — 17,400 per cu.mm .

The cells classified as monocytes were large elements, with an average cell diameters of 18.61 x 11.97 micra, and 10.07 x 7.59 micra for the nuclei. They presented abundant slightly basophilic cytoplasm; cytoplasmatic vacuoles and cells in phagocytosis were seen frequently. The nuclei had fine chromatin, sometimes reticulated, and no nucleoli were seen (figures 5 and 6) .

The PAS reaction and Sudan Black stain showed granular positivity.

DISCUSSION AND CONCLUSIONS

The values of red blood cells and hemoglobin obtained by us for the species *Mugil curema* were basically equal to the ones found by Soares (1965) for fishes of the same species in the same geographical area. We did not find hemoglobin concentrations bellow 7.0 g% ; our values were far higher than the ones found by Sulya *et al.* (*in* Thomson, 1966) .

Studying the leukocytes, we identify the lymphocytes, monocytes (macrophages according to Jakowska, 1956) , neutrophils, and thrombocytes. Soares (1965) did not refer the two latter cellular types in *Mugil curema*. Eosinophils, basophils and plasmocytoid cells were not seen in the peripheral blood of the two species studied in the present paper. These cells were found in other species of marine fishes from the brazilian northeast, reported by us (Pitombeira *et al.*, 1968 ; Pitombeira & Martins, MS).

In conclusion, no marked difference was found between the hematological data of the fishes *Mugil curema* and *Mugil incilis*. However, we have to take into consideration that the samples were rather small.

In one specimen of *Mugil curema* the leukocyte count was 5 times higher than the

average observed for twelve individuals of the same species. On the leukocyte differential count, 22% of the cells were monocytes; half of those cells had normal characters and the remainder were bigger cells, with cytoplasmatic vacuoles and/or fogocytosis.

According to Smirnova (1965) , the digestive process in fishes can elevate the leukocyte count 2 to 5 times the fasting levels. Tchistova (1967) refers authors that considered the appearence of macrophages under certain circunstances, such as period of spawning, infections, etc. Martins & Pitombeira (1968) found elevated leukocytosis in one specimen of *Scomberomorus maculatus* (Mitchill) and in two of *Opisthonema oglinum* (Le Sueur), all from the coast of the State of Ceará, suggesting the existence of blood pathology, perhaps leukemia.

Unfortunately we are unable to state the hematological changes were due to spawning period, after feeding, infection or other fish disease. It is interesting to state that the fishes were collected on polluted area. Furthermore, the leukocytes levels observed were superior to the one registered by Anderson (1957) , for the beginning of maturity in the species *Mugil curema.*

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