A hematological study on the mountain whitefish, Prosopium williamsoni
by Ida M Hubbard

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for a degree of Master of Science in Zoology
Montana State University


Abstract:
A hematological study was made on mountain whitefish (Prosopium williamsoni) collected from the Yellowstone and Madison River drainages in Montana from April 1961 to September 1962, The various blood values showed wide ranges for all fish. Comparisons were made of means for different weight, age, and sex groups. Younger fish had lower hemoglobins, hematocrits, erythrocyte sizes, leucocyte counts arid thrombocyte counts and the percentage of lymphocytes was higher than that of older fish.

Males had higher hematocrit values and more lymphocytes than females. The kidney was the most active leucopoietic tissue while the spleen was more erythropoietic.

# A HEMATOLOGICAL STUDY ON THE MOUNTAIN WHITEFISH

## *PROSOPIUM WILLIAMSONI*

by

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## ABSTRACT

A hematological study was made on mountain whitefish (*Prosopium williamsoni*) collected from the Yellowstone and Madison River drainages in Montana from April 1961 to September 1962. The various blood values showed wide ranges for all fish. Comparisons were made of means for different weight, age, and sex groups. Younger fish had lower hemoglobins, hematocrits, erythrocyte sizes, leucocyte counts and thrombocyte counts and the percentage of lymphocytes was higher than that of older fish. Males had higher hematocrit values and more lymphocytes than females. The kidney was the most active leucopoietic tissue while the spleen was more erythropoietic.

## INTRODUCTION

A hematological study on the mountain whitefish (*Prosopium williamsoni*) was conducted for the purpose of testing techniques, determining normal values, and blood cell origins. Fish were collected from the Yellowstone and Madison River drainages at all seasons during 1961-1962.

Numerous studies on fish blood show inconsistent results possibly due to techniques as reported by Lieb, *et al.* (1953), Larsen and Sneiszko (1961), and Haws and Goodnight (1962). The only study on mountain whitefish encountered was that of Bolton (1933) who studied basophil (mast) cells. However, many recent publications concern the Salmonidae (Katz, 1951; Black, 1955; and Lieb, *et al.*, 1953). Hematological investigations have also been made on various other fishes: Jordon and Spiedel (1924) studied several teleosts; Yokayama (1947) the yellow perch (*Perca flavescens*); Tyler (1960) two Antarctic fishes (*Trematomus borchgevinki* and *Notothenia larsoni*); and Young (1949) the opaleye (*Girella nigricans*)

In the present study of mountain whitefish, specimens were collected by angling and held in nets placed in the stream until transported to the laboratory. Because they were extremely sensitive to high temperatures, ice was used during the warm weather to maintain cool temperatures in the containers used for transportation. Most of the fish were brought to the laboratory before blood samples were removed, however, blood was taken from some fish at the site of capture. Some were held in laboratory troughs for varying periods without food. Prior to taking blood samples, fish were rapidly anesthetized with a 1:20,000 dilution of tricaine methane sulfonate (MS 222). Each fish was measured, weighed and sexed and scales were removed for age determinations. Some fish were kept alive following the removal of blood. These were individually tagged and held in troughs for various periods for subsequent sampling.

A laboratory test exposing fish to varying dissolved oxygen conditions during a two hour period and involving anesthetized and unanesthetized fish showed no effect on blood values.

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## RESULTS

### Hemoglobin

Anticoagulated blood was used for hemoglobin determinations. Because fish blood clots very rapidly, it was important to use an anticoagulant that prevented clotting yet caused no hemolysis of erythrocytes. By rinsing the syringe with a 10 percent solution of dipotassium ethylene-diamine tetra-acetate (EDTA) and expelling all excess it was possible to obtain a satisfactory blood sample. The dry chemical did not dissolve readily with fish blood. Wintrobe (1962) reported on the use of various anticoagulants and recommended EDTA for human hematology. Heparin also has been used satisfactorily as an anticoagulant by many investigators. It did not prevent clotting as completely as EDTA when mixed with mountain whitefish blood.

Blood was removed from the caudal artery by the use of a syringe (needle size 22). The needle was inserted anteriorly until it struck the vertebral column at a $45^{0}$ angle just posterior to the anal fin. It was then retrieved about one millimeter until a steady flow of blood was obtained. Approximately 0.5 ml was taken from fish (40-85 g) and 1-2 ml from larger fish.

A comparative hemoglobin study was done on six fish using oxyhemoglobin, acid hematin, and cyanmethemoglobin methods (Table I). These were used on each blood sample. Dilutions were made with the same 0.02 ml pipette thus eliminating variations due to differences in pipettes. Two to three determinations were performed for each method and the mean recorded. A Klett-Summerson photoelectric colorimeter was used for all hemoglobin tests except those done with the acid hematin technique by the Sahli method. Ortho's Aculobin Hemoglobin standard was used to make

TABLE I

Hemoglobin values (g/100 ml blood) determined by various methods.

| Sample number | Cyanmet-hemoglobin | Oxyhemo-globin | Acid hematin Klett-Summerson | Acid hematin Sahli |
|---|---|---|---|---|
| 1 | 11.61 | 11.57 | 14.12 | 13.67 |
| 2 | 7.52 | 7.67 | 9.90 | 10.13 |
| 3 | 8.32 | 8.29 | 10.95 | 10.80 |
| 4 | 9.76 | 9.52 | 12.33 | 11.70 |
| 5 | 8.28 | 8.50 | 10.90 | 10.10 |
| 6 | 5.65 | 5.88 | 8.65 | 8.40 |

standard curves. This was found to be an accurate and practical method of calibrating the colorimeter. Cyanmethemoglobin determinations were made with dilutents prepared with Ortho's pellets. Comparisons between the use of Drabkin's solution and the Ortho dilutent showed both fluids to be satisfactory for fish blood hemoglobins. I preferred the pellets because of the ease with which solutions were prepared. Wintrobe (1962) reported that the cyanmethemoglobin method is accurate. It was used satisfactorily with fish blood by Sinderman et al. (1961). Because erythrocyte nuclei were not destroyed when acid hematin methods were used, these were considered unsatisfactory. The oxyhemoglobin technique gave similar results to those of cyanmethemoglobin.

Hemoglobin ranges and means were determined on 119 mountain whitefish (ages 1-6 years) collected from April 1961 to September 1962 (Table II). The range was 7.0-13.7 g per 100 ml blood with a mean of 10.6 g. Six fish taken in April 1961 had a mean of 12.8 g, however this may have resulted from an inadequate sample. A comparison based on weights (Table III)

TABLE II

Hemoglobin and hematocrit determinations; erythrocyte, leucocyte, and thrombocyte counts on mountain whitefish taken at various times. No. fish in parentheses. Range = R, Mean = M.

| | Hemoglobin gram | | Hematocrit percent | | Erythrocyte count million | | Leucocyte count thousand | | Thrombocyte count thousand | |
|---|---|---|---|---|---|---|---|---|---|---|
| | R | M | R | M | R | M | R | M | R | M |
| April 1961 | 10.8-13.7 | 12.8 (6) | 48.0-54.7 | 52.0 (5) | 1.81-2.20 | 2.05 (4) | | | | |
| July-Sept. 1961 | 7.0-12.9 | 10.4 (32) | 31.7-57.2 | 41.1 (30) | 1.12-1.87 | 1.35 (29) | 0.83-25.5 | 7.1 (32) | 3.3-35.2 | 17.8 (32) |
| July-Sept. 1962 | 9.6-13.0 | 11.1 (18) | 33.2-62.2 | 50.0 (15) | 1.03-1.46 | 1.28 (15) | 1.5-15.9 | 6.5 (14) | 5.4-35.2 | 17.0 (15) |
| Oct.-Dec. 1961 | 7.8-12.8 | 10.3 (38) | 28.5-54.0 | 41.9 (40) | 1.01-1.99 | 1.87 (38) | 0.66-20.6 | 5.3 (37) | 7.9-47.2 | 19.9 (38) |
| Jan.-Mar. 1962 | 7.8-12.7 | 10.5 (25) | 30.7-52.8 | 43.2 (24) | 1.04-2.34 | 1.46 (23) | 0.36-5.6 | 2.1 (20) | 0.87-28.0 | 13.2 (21) |
| Total | 7.0-13.7 | 10.6 (119) | 28.5-59.3 | 43.5 (114) | 1.01-2.34 | 1.57 (109) | 0.36-25.5 | 5.4 (103) | 0.87-47.2 | 17.6 (106) |