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Journal Title: Journal of Fish Biology
Volume: 5
Issue: 6
Month/Year: , 1973
Pages: 771-781
Article Author: , Blaxhall P. C.
Article Title: Routine haematological methods for use with fish blood
ISSN: 0022-1112
OCLC #: info:doi/10.1111/j.1095-8649.197

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J. Fish Biol. (1973) 5, 771–781

Routine haematological methods for use with fish blood

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(*Received 14 May 1973*)

Some routine haematological methods for examining fish blood are described including haemoglobin estimation, haematocrit, erythrocyte counts, erythrocyte sedimentation rate, total and differential leukocyte counts, and cytochemical staining. Descriptions of stained blood cells are given as are the ranges and mean values for the above tests on brown trout *Salmo trutta* (L.). These methods are suggested as a possible means of assessing fish health but there is a need for establishing values in health, disease and various stress conditions before their value in diagnosis can be evaluated.

I. ROUTINE HAEMATOLOGICAL METHODS FOR USE WITH FISH BLOOD

Many routine haematological methods already exist in human medicine to assist in providing evidence, and possible identification, of an abnormality or disease process. Some of the methods could readily be adapted for use with fish blood as has indeed been done by some workers (Blaxhall, 1972). It was therefore decided to establish some routine haematological screening methods with healthy fish which may assist in assessing fish health.

The tests selected were as follows:

(a) tests for the presence of anaemia; in these were included haemoglobin estimation, erythrocyte counts, haematocrit value, and the examination of a Romanowsky stained film;

(b) Erythrocyte sedimentation rate (ESR), which is a non-specific reaction indicating the presence and intensity of a disease process;

(c) total leukocyte and differential leukocyte counts as an indication of the presence and possible type of an infectious or organic disease;

(d) cytochemical stains to aid in the identification of the less mature cells which may be found in fish blood.

The fish used in establishing these methods were 73 hatchery-reared brown trout *Salmo trutta* (L.), approximately two years old, and weighing about 160 g. The water supplying the hatchery was from a borehole (Table I) and the temperature in the ponds varied seasonally in the range 7–16° C. The fish diet was a mixture of pelleted food and fresh meat. All the tests were carried out on blood taken from the fish by cardiac puncture, and apart from the staining of the films, the tests were completed on the day of collection. The films were suitably fixed and stained later.

BLOOD SAMPLING TECHNIQUE

In order to minimize the possible variations in the blood values, the technique was standardized as follows. The fish were caught gently in a small net, avoiding stress

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as much as possible, and immediately anaesthetized in MS222 (Sandoz) using a concentration of 1/15000. The time taken for the fish to be anaesthetized was usually about 2–3 min, as shown by loss of equilibrium and by immobility when touched. When this occurred the fish was placed on its back in a V-section trough and blood taken by cardiac puncture using a 2 cm^3 sterile plastic syringe and a No. 21 swg needle. The use of plastic syringes is a necessary precaution with fish blood because contact with glass results in shortened coagulation times (Smith, Lewis & Kaplan, 1952). The site chosen for the puncture was about half an inch behind the apex of the 'V' formed by the gill covers and isthmus; Klontz & Smith (1968) described the anatomical landmarks. To avoid contamination with mucus and water the area was wiped dry with a tissue. The needle was inserted at right angles to the surface of the fish and was very slightly aspirated during penetration. It was then pushed gently

TABLE I. Analysis of borehole water supplying the brown trout hatchery

B.O.D. (5 days at 20° C)	1·1 mg/l
Total suspended solids	1·0 mg/l
Nitrogen as—ammonia	0 mg/l
nitrite	0·1 mg/l
nitrate	8·2 mg/l
Chlorides as Cl	36 mg/l
Alkalinity as $CaCO_3$	86 mg/l
Total hardness as $CaCO_3$	185 mg/l
Conductivity (25° C)	410 micromhos
pH	8·3
Anionic detergent	0 mg/l
Cadmium	0 mg/l
Chromium	0 mg/l
Copper	0·02 mg/l
Nickel	0 mg/l
Zinc	0 mg/l

down until blood started to enter as the needle punctured the heart. Blood was taken under gentle aspiration until 0·5 cm^3 had been obtained, then the needle was withdrawn. After detaching the needle from the syringe, the blood was mixed well in a vial containing anticoagulant (potassium salt of ethylenediamine tetra-acetic acid, EDTA) to give a final concentration of 5 mg EDTA per cm^3 blood. This anticoagulant was chosen as being currently used in routine haematological investigations in human medicine and giving the best results with preserved blood. During this time the assistant smeared some of the fish mucus over the puncture site to minimize the risk of infection being established, and then the fish was placed in water to recover consciousness, which normally took about 5–10 min. Klontz & Smith (1968) have dealt with various aspects of anaesthesia and recovery in fish.

ERYTHROCYTE SEDIMENTATION RATE

As the volume of blood available was small, methods using capillary tubes were developed. Microhaematocrit tubes of 1·1–1·2 mm internal diameter and 75 mm long were used. Two methods were compared, both being adaptations of the standard

Wintrobe and Westergren methods (Britton, 1963). Preliminary studies using human blood gave consistently reproducible results comparing the standard Westergren with the micro-Westergren method. Although there is a noticeable fall in velocity with the micro method, the test was able to distinguish between human blood from healthy and diseased persons. In human medicine the ESR is a non-specific reaction giving a measure of the presence and intensity of disease processes in the body. It is usually raised with increased tissue destruction as in acute infections, heavy-metal poisoning, nephritis, etc. Schumacher, Hamilton & Longtin (1956) used a variation of the standard Westergren method in testing brook trout *Salvelinus fontinalis* (Mitchill) with furunculosis, and found diseased fish always had raised rates of fall. Barnhart (1969) used heparinized blood and a micro-Wintrobe method in measuring the ESR of rainbow trout *Salmo gairdneri* (R.). The two methods adapted for use with brown trout *Salmo trutta* (L.) blood were those sometimes used in paedriatic medicine. In the micro Wintrobe method, anticoagulated blood was allowed to flow into the tube for about 50 mm, one end sealed with ' Critaseal ' and the tube stood in the vertical position for 1 h at room temperature. The reading was made with the aid of millimetre graph paper mounted on card. Although the rate of fall is not as great as with the micro-Westergren method it has the advantage of allowing the tube to be used subsequently for microhaematocrit determinations and also corrections factors for anaemia could be worked out and applied as in human medicine. The micro-Westergren method involved mixing the anticoagulated blood with 1/5 of its volume of 3·8% sodium citrate before being introduced into the tube to about 50 mm depth. The tube was sealed and left in the vertical position for 1 h before being read. The measurement is made from the top of the column of sedimented erythrocytes to the surface of the plasma. The tubes must be kept absolutely vertical otherwise the rate of sedimentation is affected. The results of both methods with clinically healthy brown trout *Salmo trutta* (L.) are given in Table II. Diseased fish were not available for comparison.

TABLE II. Haematological values for healthy brown trout. 73 fish were sampled. The water temperature varied seasonally between 7–16° C

Parameter	Range	Mean	±S.E.
Haemoglobin g/100 cm³	4·1–10·3	6·83	1·48
Erythrocytes/mm³	0·606–1·320	0·995	0·160
Haematocrit %	20–43	32	4·88
ESR Westergren mm/h	1–5	2·6	0·7
ESR Wintrobe mm/h	0·5–2·5	1·2	0·4
Leukocytes/mm³	2000–63000	11536	9061
Lymphocytes %	56–100	90	8·9
Neutrophils %	0–25	6·6	6·5
' Metamyelocytes ' %	0–11	1·4	2·3
' Myelocytes ' %	0–8	1·6	1·9
Blasts %	0–4	0·3	0·7

HAEMOGLOBIN ESTIMATION

Various methods for the measurement of haemoglobin in fish blood have been used. When comparing the methods using trout blood, Larsen & Snieszko (1961) found the cyanmet-haemoglobin method superior because of the consistency of the

results, stability of the reagents and the commercial availability of standards. After thoroughly mixing the blood, 0·02 cm^3 was placed into 4 cm^3 of Drabkin's reagent. The solution was gently mixed by inversion and allowed to stand for at least 10 min for full conversion of haemoglobin to cyanmethaemoglobin. The small coagulum which formed was removed with a wooden stick. Transmittance was read on an EEL spectrophotometer at a wavelength of 540 nm and this reading was converted to haemoglobin concentration in grams per 100 cm^3 by reference to a graph constructed using commercially available cyanmethaemoglobin standards

Drabkin's Reagent	
Potassium ferricyanide	20 mg
Potassium cyanide	50 mg
Distilled water	1 l

This is commercially available in powder or tablet form. This should be stored at room temperature in a bottle excluding all light where it will keep for several months. This method will convert haemoglobin, methaemoglobin and carboxyhaemoglobin into cyanmethaemoglobin, but will not convert sulphaemoglobin. Table II gives the figures obtained with healthy brown trout *Salmo trutta* (L.).

TOTAL ERYTHROCYTE AND LEUKOCYTE COUNTS

Three diluting fluids were tried: Shaw's (1930), Rees-Ecker fluid (Lucas & Jamroz, 1961), and Dacie's fluid (Dacie & Lewis, 1968). The latter fluid stored well and stained the cell nuclei effectively when brilliant cresyl blue was added. The generally accepted use of bulb-type diluting pipettes was discontinued after finding that the resulting dilution factor for the blood was inconveniently large therefore giving a high coefficient of variation, and also that the stain was difficult to remove from the pipettes. The method adopted was to make a 1 in 50 dilution of the blood by measuring 20 mm^3 of blood with a Sahli pipette into 0·98 cm^3 of diluent. The suspension was introduced into an improved Neubauer counting chamber and the cells counted by eye. For the erythrocyte count 1/5 mm^2 was counted, while for the leukocyte count 4 mm^2 or if the numbers appeared low then 9 mm^2 were counted. The use of an electronic cell counter would greatly improve the accuracy of the counts but this is not feasible because the erythrocyte nuclei cannot be lysed to facilitate leukocyte counts.

Modified Dacie's fluid	
40% Formaldehyde	10 cm^3
Trisodium citrate	31·3 g
Brilliant cresyl blue	1 g
Distilled water	1 l
Filter before use	

MICROHAEMATOCRIT (PACKED CELL VOLUME)

The well mixed blood was drawn into a microhaematocrit tube (Hawkesley Ltd.) 75 mm long, 1·1–1·2 mm internal diameter and one end was sealed with ' Critaseal '. The tube was then centrifuged in a microhaematocrit centrifuge for 5 min at 10 500 rev/min. The readings were made with the aid of a microhaematocrit reader and expressed as the volume of the erythrocytes per 100 cm^3. This procedure is widely

used in human haematology and its value in fishery research and management reported (Snieszko, 1960).

GENERAL CYTOLOGICAL AND CYTOCHEMICAL STAINING OF BLOOD FILMS

As a differential blood cell count is invaluable to the haematologist in aiding the diagnosis of a wide range of human diseases, it was thought advisable to include this in the examination of the fish blood. Normal values need establishing before comparisons can be made. For the general cytological staining, thin blood films, after being allowed to dry, were fixed in methanol and then stained by the May Grunwald–Giemsa method (Hayhoe, Quaglino & Doll, 1964). This stain is considered by many medical haematologists to give results superior to other Romanowsky stains. A differential cell count, while being valuable clinically, does require experience in recognition and classification of the cells which probably accounts for the reason why this procedure has not been fully developed in fish haematology. In order to assist in this identification it was considered desirable to introduce the use of cytochemical stains which are generally used in human medicine as an aid in identifying cells. Hayhoe *et al.* (1964) described their use in human medicine and also devised a system of scoring and assessment of the cells which could be adapted for use with fish blood if necessary.

Sudan black B stain. The method used was that of Sheehan & Storey (1947). Thin blood films were fixed in a solution of 10 cm^3 formalin and 90 cm^3 ethanol for 5 sec before being washed for 10 min in running water. Two differing forms of positivity in leukocytes have been shown. The first, occurring in granulocytes, appears as a faint localised cytoplasmic sudanophilia in the least mature cells and increases rapidly to strong localised or heavy overall positivity as the cells mature. The second is found in only a proportion of monocytic cells (human), and consists of discrete sudanophilic granules in both cytoplasm and nucleus alike.

Periodic acid Schiff reaction. This technique was a modification of McManus (1946). Hayhoe, Quaglino & Flemas (1960) reported that blood films up to 10 years old and already stained by Romanowsky will give good results.

Positivity is shown by a magenta colouration. Granulocytic cells progress from negative blast cells to strongly positive mature neutrophils, with intermediate degrees of both diffuse and granular positivity in cells of intermediate maturity; lymphocytic cells occasionally show positive granules, varying in number and size against a negative cytoplasmic background; erythrocytes are entirely negative.

Peroxidase reaction. The method of Sato & Sekiyo (1926) was tried with brown trout *Salmo trutta* (L.) blood, but it was found that the method of Kaplow (1965) gave better results. For this stain thin blood films were fixed for 1 min in a solution of 10 cm^3 formalin and 90 cm^3 ethanol. Peroxidase granules appear intensely black. Granulocytic cells from the myeloblastic stage onwards show cytoplasmic activity increasing in intensity as cell maturation progresses. All other cell types are negative but human monocytes occasionally show a weak reaction.

The appearance of peripheral blood cells of brown trout *Salmo trutta* (L.) stained by cytochemical methods is as follows.

Granulocyte series with a range from strongly positive granular and diffuse PAS positivity in the mature neutrophil, to a diffuse positivity with a few granules in the ' myelocyte '.

The peroxidase reaction showed strongly positive coarse granules in the neutrophil, a few strongly positive granules in the ' myelocyte ' and varying intermediary degrees between.

Sudan black B staining varied from very heavy, coarse cytoplasmic granules tending to obscure the nucleus in neutrophils, through intermediary grades to diffuse positivity with a few strongly positive granules present in the ' myelocyte '. The less mature cells displayed fewer granules and a less intense diffuse staining of the cytoplasm. It must be admitted that much confusion still exists over the nomenclature of these cells. ' Myeloid ' cells are so called for descriptive convenience.

Lymphocytic series of cells which are not stained by Sudan black and the peroxidase reaction showed in some cells small blocks of PAS positive granules in the cytoplasm.

Erythrocytes and thrombocytes were negative with all the cytochemical methods used.

Trout blood cells stained by Romanowsky stains are described by various authors: Catton (1951), Agnesotti (1932), Barnhart (1969) and Weinreb & Weinreb (1969). Useful descriptions of the peripheral blood cells of the common goldfish *Carassius auratus* (L.) are given by Watson *et al.* (1963) and Weinreb (1963). Weinreb states that the goldfish represents one of the most highly differentiated blood pictures among the teleosts.

Peripheral blood cells of brown trout *Salmo trutta* (L.) stained by May-Grunwald-Giemsa stain in this present work are as follows; cell sizes are given in Table III.

TABLE III. Sizes of cells in healthy brown trout *Salmo trutta* (L.) as seen in a random selection of 25 May-Grunwald-Giemsa stained blood films selected from a sample of 73 different fish

Cell type	No. measured	Range (μm)	Mean (μm)
Erythrocyte	200	10·8–18·0×9	15·2×9
Small lymphocyte	224	5·4–8·1	7·0
Large lymphocyte	15	8·1–10·8	9·1
Neutrophil	128	7·2–13·5	9·1
' Metamyelocyte '	20	9·9–12·6	9·7
' Myelocyte '	24	7·2–12·6	10·7
Blast cell	5	12·6–15·3	15·1

Erythrocyte. Ellipsoidal cells measuring 15·2×9 μm with the nucleus centrally located with its long axis parallel to that of the cell, the densely clumped chromatin staining purple and having alternate lighter and darker areas. In the fully haemoglobinized cell the cytoplasm stained a buff colour. The less mature cells, polychromatocytes, numbered about 1% of the erythrocytes in the healthy fish. These were smaller and more rounded in shape with the cytoplasm assuming a polychromatic greyish-blue colour. The nucleus is more rounded than in the mature cell and the chromatin has a more open appearance.

Lymphocytic series. The lymphocyte is the next most numerous cell to the erythrocyte in fish blood. The size of the lymphocyte varies according to its stage of maturity, as does its cytoplasmic : nuclear ratio. The nucleus occupies more of the cytoplasm as the cell matures. An occasional azurophilic granule may be seen in the cytoplasm. In this study small lymphocytes were not differentiated from large lymphocytes.

Large lymphocytes. These cells are the precursors of the small lymphocyte and measure about 9·1 μm. The nucleus tends to be eccentric, occupying about 3/4 of the cytoplasm, the chromatin staining dark purple and fairly homogeneous in appearance. The cytoplasm was nongranular and stained dark blue. The nucleus in a small number of the cells had an indentation facing the centre of the cytoplasm.

Small lymphocyte. This cell accounted for most of the lymphocyte population. It is a round cell, measuring about 7 μm. The nucleus occupies most of the cell leaving a thin rim of clear blue cytoplasm. The chromatin of the nucleus is a compact mass staining dark purple with lighter areas of interchromatic material.

Granulocytic series. Only cells of the neutrophilic series were seen in brown trout *Salmo trutta* (L.). In common with the findings of Catton (1951) the granules in the neutrophils did not stain as well as they do with human cells. As these cells are well documented in human medicine it was felt advisable to adopt as closely as possible that nomenclature (McDonald, Dodds & Cruickshank, 1970).

Polymorphonuclear neutrophils. The average size of these cells was about 9·1 μm. The nucleus consists of dense masses of purple staining chromatin; the typical lobation and thin chromatins strands found in human cells was seen in only a few cells in brown trout *Salmo trutta* (L.). Usually the nucleus seemed more bulky and assumed a 'ribbon' or 'sack-like' form, often convoluted with parts overlaying each other thus making it resemble a monocyte. A very few cells did possess nuclei with up to five nuclear lobes; this was not common with brown trout *Salmo trutta* (L.), but the incidence of the lobated feature is thought to vary with the species. The cytoplasm stains a pale pinkish colour and has a granular appearance without the typical granules found in human cells.

'*Metamyelocytes.*'† These measured about 9·7 μm, having a cytoplasm similar to that of the polymorphonuclear cell but being slightly bluer in colour. The nucleus stains purple, contains strands of chromatin and has an indentation facing the centre of the cell. The nucleus occupies about 4/5 of the area of the cell.

'*Myelocytes.*'† This cell measured about 9·7 μm, having a rather irregularly staining bluish-grey cytoplasm with a granular appearance but no definite granules visible. The nucleus was round, eccentric, sometimes slightly indented and occupied about 3/4 of the cell. The nucleus stained red-purple, with definite chromatin strands but no nucleoli visible.

Blast cells. Primitive cells of each cell series could not be differentiated with any degree of certainty and being too few in numbers to differentiate by cytochemical staining, they were included under the general term of blast cells. The average size of these cells was 15·1 μm. The nucleus occupied about 4/5 of the cell, the chromatin staining a lighter purple and having a stippled effect. Occasionally indistinct nucleoli were visible. The cytoplasm was an intense basophilic blue and lacked granules. Catton (1951) and Weinreb & Weinreb (1969) describe such primitive cells in brown trout *Salmo trutta* (L.) and goldfish *Carassius auratus* (L.). Weinreb & Weinreb (1969) considered that because of the great overlap in morphological criteria for the identification of the more primitive cells found in fish blood, it is more advantageous to group the haemocytoblast, lymphoid hemoblast and lymphoblast as 'blast cells'. They also found that most of the blast cells were lymphoblasts.

Monocytes were not identified in brown trout *Salmo trutta* (L.), though some of the

† The term 'metamyelocyte' and 'myelocyte' have been used because of the close morphological similarity of these cells in trout with the well documented human cells of the same terminology.

neutrophils and metamyelocytes could easily be mistaken unless cytochemical staining methods are used.

Thrombocytes were variable in shape, sometimes round, elongated or 'flask-shaped'. The structure and staining characteristics of the nucleus resembles that of the lymphocyte. The cytoplasm stains a pale blue colour and the cell gives the general appearance of a badly distorted lymphocyte.

II. DISCUSSION

Many workers have described the use of isolated haematological parameters on fish blood, as reviewed in a previous paper (Blaxhall, 1972). The need for establishing normal haematological values in fish with a view to aiding in the diagnosis of disease (Hesser, 1960; Larsen & Snieszko, 1961; Snieszko, 1960; Summerfelt, 1967) and in connection with pollution and its effects (Mawdesley-Thomas, 1971) has been emphasized. It would seem appropriate to develop some standardized screening tests of a routine nature which could be applied to fish blood. As such tests already exist in human medicine evidently the most straightforward approach is to adapt these for use with fish blood. The tests chosen involve the use of less than 0·5 cm^3 blood which means the fish do not necessarily need to be killed. It should be remembered that these are only screening tests and as in human medicine need correlating with the clinical condition and preferably biochemical tests of the subject. Much work still remains to be done before full use can be made of haematological investigations, especially the establishment of normal values together with the identification of those environmental and disease conditions that may affect these values. If workers used these screening tests whenever possible in their investigations a bank of useful information would soon be available from which valuable and informative conclusions could be drawn. Various derived values can also be calculated from the results of these haematological tests, such as mean cell volume, mean cell haemoglobin concentration and absolute differential leukocyte numbers. In this present work only healthy brown trout *Salmo trutta* (L.) were available for testing, but other workers have found altered haematological values in fish blood, among which was a raised ESR in furunculosis of brook trout *Salvelinus fontinalis* (Mitchill) (Schumacher *et al.*, 1956). Kawatsu (1969), found a brook trout *Salvelinus fontinalis* (Mitchill) suffering from a macrocytic anaemia. Using rainbow trout *Salmo gairdneri* (R.), Weinreb (1958) made the observation that the mechanism of leukocyte control and physiological response of each cell type seems comparable to that of the mammal. If this is so then the leukocyte total and differential count could occupy as important a place in fish studies as it does in human medicine. Apart from the use of the peroxidase stain on the blood of rainbow trout *Salmo gairdneri* (R.) by Yuki (1958), cytochemical methods have not been greatly used. He sought to use it as a biological indicator to show a fluctuation in the ratio of positive to negative cells. Haemoglobin estimation has been widely used in fish studies, but with such a variety of methods it is difficult to correlate the results. Summerfelt (1967) using goldfish *Carassius auratus* (L.) found sexual variation but gave the mean as 8·3 g/100 cm^3. Using the cyanmethaemoglobin method on pike *Essox lucius* (L.), Mulcahy (1970) gave a range of 5·6–15·0 g/100 cm^3 with a mean of 8·8 g/100 cm^3. Larsen & Snieszko (1960) using the same method give the following mean values: brown trout *Salmo trutta* (L.) 8·8 g/100 cm^3, brook trout *Salvelinus fontinalis* (Mitchill) 9·8 g/100 cm^3, and rainbow trout *Salmo gairdneri* (R.) 9·6 g/100 cm^3; whereas Schiffman & Fromm (1959) gave

a value of 6·5 g/100 cm³ for rainbow trout *Salmo gairdneri* (R.) using the acid haematin method. While the above values using the cyanmethaemoglobin method are higher than those obtained in the present work they do fall within the range of values obtained and show the variation which may be expected, possibly reflecting the age and nutritional state of the fish.

Erythrocyte sedimentation rate has not been widely used on fish blood and most workers have used a micro-Wintrobe method with heparinised blood. Murachi (1959) using carp *Cyprinus carpio* (L.) reported values of 0·1–4·6 mm/h, and Barnhart (1969) using rainbow trout *Salmo gairdneri* (R.) gave a range of 0·5–21 × 0·7 mm/h which seems excessively raised. Using rainbow trout *Salmo gairdneri* (R.), Sano (1960) gives values of 0·2–1·6 mm/h. Schumacher *et al.* (1956) using a Westergren type method gave values of 10–28 mm/h in brook trout *Salvelinus fontinalis* (Mitchill) suffering from furunculosis as compared to 4–8 mm/h with healthy fish. In the present work low values were obtained with healthy brown trout *Salmo trutta* (L.), but as high values have been obtained with blood from diseased humans using the same micro methods it would seem most likely that such results could be obtained with various disease conditions of fish. The haematocrit has been suggested as of use in fishery management in checking for anaemia (Snieszko, 1960). Sex differences in the values of mature healthy fish were found, and the need for two standards were proposed: (a) general, and (b) individual hatchery. He recorded values for brown trout *Salmo trutta* (L.) 39–44%, brook trout *Salvelinus fontinalis* (Mitchill) 45–50% and rainbow trout *Salmo gairdneri* (R.) 45–53%. The values obtained in the present work accord with the above figures except that they extend to a much lower figure possibly reflecting the fact that they incorporate seasonal variations. Sano (1960) found variation in rainbow trout *Salmo gairdneri* (R.) with gonad development, the male varying between 22–46% and the female 19–38%. Schiffman & Fromm (1959) gave a mean value of 31·8% with rainbow trout *Salmo gairdneri* (R.). Using adult pike *Essox lucius* (L.), Mulcahy derived values of 20–43·5% with a mean of 32%.

Erythrocyte counts suffer from a fair degree of inherent error when carried out visually, and so greater reliance is placed on haematocrit and haemoglobin estimation as indicators of anaemia. The values obtained for brown trout *Salmo trutta* (L.) obtained in the present work accord fairly well with those of Snieszko (1960). He gives values of 1 180 000/mm³ for brown trout *Salmo trutta* (L.), 1 370 000/mm³ for rainbow trout *Salmo gairdneri* (R.), and 1 260 000/mm³ for brook trout *Salvelinus* fontinalis (Mitchill).

Leukocyte counts seem to have a wide range of variation 2000–63 000/mm³ in the present work, though the 95% confidence limits for brown trout *Salmo trutta* (L.) are 0–29 295/mm³. Other workers have found similar variation, Catton (1951) gives 23 000–46 000/mm³ for brown trout *Salmo trutta* (L.) and roach *Rutilis rutila* (L.), while for pike *Essox lucius* (L.), Mulcahy (1970), gives 79 000–13 7000/mm³. More work needs doing on the factors affecting leukocyte levels and it is possible that a change in the relative proportions of the different cells present could be used as an indicator of some disease or pollution process upon the fish. Most work concerning differential cell counts seems to have been carried out on goldfish *Carassius auratus* (L.) (Watson *et al.*, 1963; Weinreb & Weinreb, 1969). Weinreb (1958) used leukocyte count changes as a means of assessing the systemic response of rainbow trout *Salmo gairdneri* (R.) to various injections. At present there is not sufficient evidence to prove the usefulness of leukocyte differential counts on fish; such evidence will

accumulate if the test is increasingly used by interested workers and then it could prove its diagnostic utility as it has in human medicine. Measurements of the cells of brown trout *Salmo trutta* (L.) agree fairly well with those of Catton (1951) who gives lymphocytes as 5–10 μm neutrophils as 9–12 μm and erythrocytes as 11×8 μm. With all these tests, the information derived needs relating to the experimental conditions, i.e. species, age and source of the fish, diet, water temperature, sampling and testing techniques.

III. CONCLUSIONS

Some routine haematological methods for examining fish blood are described including haemoglobin estimation, haematocrit, erythrocyte counts, erythrocyte sedimentation rate, total and differential leukocyte counts and cytochemical staining. The ranges and mean values for these tests on brown trout *Salmo trutta* (L.) are given. The physiological normal ranges for some of the tests are wider than those in human medicine and show the need for establishing values for fish in healthy, diseased and various stress conditions in order that their use as an aid in diagnosis may be evaluated.

This work was carried out at Trent Polytechnic, Nottingham. We thank the Director of the Polytechnic and Dr R. F. Stock for allowing the facilities to carry out this work. We would express our appreciation to Mr R. Lloyd, Ministry of Agriculture, Fisheries and Food Laboratories, London for his critical reading of the manuscript; and also to the Trent River Authority and especially Mr W. Vaughan for supplying the fish.

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JOURNAL OF FISH BIOLOGY

Volume 5
1973

Published for the Fisheries Society of the British Isles by Academic Press, London and New York

Printed in Great Britain by Henry Ling Ltd., at the Dorset Press, Dorchester, Dorset