

LOAN COPY ONLY

MICHU-T-85-001 C2

**IMPACT OF SEA LAMPREY  
PARASITISM ON THE BLOOD  
FEATURES AND HEMOPOIETIC  
TISSUES OF RAINBOW TROUT**



**CIRCULATING COPY**  
Sea Grant Depository

NATIONAL SEA GRANT DEPOSITORY  
PELL LIBRARY BUILDING  
URI, NARRAGANSETT BAY CAMPUS  
NARRAGANSETT, RI 02882

**Great Lakes Fishery Commission**

**TECHNICAL REPORT No. 46**

Michigan Sea Grant College Program  
MICHU-SG-87-308

The Great Lakes Fishery Commission was established by the Convention on Great Lakes Fisheries between Canada and the United States, which was ratified on October 11, 1955. It was organized in April 1956 and assumed its duties as set forth in the Convention on July 1, 1956. The Commission has two major responsibilities: first, develop coordinated programs of research in the Great Lakes and, on the basis of the findings, recommend measures which will permit the maximum sustained productivity of stocks of fish of common concern; second, formulate and implement a program to eradicate or minimize sea lamprey populations in the Great Lakes.

The Commission is also required to publish or authorize the publication of scientific or other information obtained in the performance of its duties. In fulfillment of this requirement the Commission publishes the Technical Report Series, intended for peer-reviewed scientific literature, and Special Publications, designed primarily for dissemination of reports produced by working committees of the Commission. Technical Reports are most suitable for either interdisciplinary review and synthesis papers of general interest to Great Lakes fisheries researchers, managers, and administrators or more narrowly focused material with special relevance to a single but important aspect of the Commission's program. Special Publications, being working documents, may evolve with the findings of and charges to a particular committee. Sponsorship of Technical Reports or Special Publications does not necessarily imply that the findings or conclusions contained therein are endorsed by the Commission.

## COMMISSIONERS

### *Canada*

P. S. Chamut  
J. C. Davis (nominee)  
A. S. Holder (nominee)  
H. A. Regier

### *United States*

W. P. Horn  
W. M. Lawrence  
J. M. Ridenour  
C. Ver Duin

## SECRETARIAT

C. M. Fetterolf, *Executive Secretary*  
A. K. Lamsa, *Assistant Executive Secretary*  
R. L. Eshenroder, *Senior Scientist for Fishery Resources*  
M. A. Ross, *Fishery Biologist*  
B. S. Staples, *Administrative Officer*  
R. E. Koerber, *Word Processing Supervisor*  
A. B. Broda, *Secretary*

**IMPACT OF SEA LAMPREY  
PARASITISM ON THE BLOOD  
FEATURES AND HEMOPOIETIC  
TISSUES OF RAINBOW TROUT**

by

**RONALD E. KINNUNEN**  
Michigan Sea Grant  
U. P. Extension Center  
1030 Wright Street  
Marquette, MI 49855

and

**HOWARD E. JOHNSON**  
Office of the Governor  
Capitol Station  
Helena, MT 59620

**TECHNICAL REPORT NO. 46**

Great Lakes Fishery Commission  
1451 Green Road  
Ann Arbor, Michigan 48105

June 1985

## CONTENTS

Abstract .....	1
Introduction .....	1
Methods and materials .....	2
Experimental animals .....	2
Experimental procedure for wound development .....	2
Hematology procedure .....	2
Histopathologic technique .....	3
Experimental procedure for wound healing .....	3
Results and discussion .....	3
Blood features of wound developing and wound healing fish .....	3
Blood features of mortally wounded fish .....	8
Histopathology of the kidney and spleen .....	11
Appendix .....	13
References .....	15

## ABSTRACT

Rainbow trout (*Salmo gairdneri*) held in the laboratory were subjected to sea lamprey (*Petromyzon marinus*) attack for prescribed time periods. Blood and hemopoietic tissue samples taken during wound development and wound healing provided information on the hosts' ability to recover after a lamprey attack. The blood features that were monitored included the hematocrit, hemoglobin, red blood cell precursors, leucocrit, and leucocyte differential. The spleen was the only hemopoietic organ which exhibited pathological change. During lamprey attachment the hematocrit and hemoglobin values for host fish were higher than for control fish, which was attributed in part to a stress response and the inability of the smaller sized lamprey (19.1 cm and 13.5g) to induce anemia. There was no significant change in the red blood cell precursors during this wound development period, and host fish demonstrated lymphopenia with concomitant neutrophilia. The hematocrit and hemoglobin values for fish in the wound healing group dropped significantly at 2 days following lamprey detachment, which is believed to be attributed to hemodilution via the wound area. The number of red blood cell precursors rose significantly during wound healing and reached a peak value at 1 month. Lymphopenia with concomitant neutrophilia was evident 2 days following lamprey detachment.

## INTRODUCTION

The feeding mechanism of sea lamprey (*Petromyzon marinus*) is adapted for obtaining liquid food, principally blood sucked from the host fishes (Lennon 1954). Body fluids enter the lampreys' diet to a lesser degree and a considerable amount of reduced flesh, particularly muscle, is also ingested. Within the confines of the wound the capillaries are destroyed and blood and lymph are ingested by the lamprey. Lennon also found that the buccal gland secretions from sea lamprey prevented fish blood coagulation.

The daily blood consumption by sea lamprey at  $10 \pm 1^\circ\text{C}$  feeding on rainbow trout (*Salmo gairdneri*) and lake trout (*Salvelinus namaycush*) ranged from 2.9 to 29.8% (average,  $11.6 \pm 7.5\%$ ) of the lamprey's wet body weight per day (Farmer et al. 1975). They found the hematocrits of dying fish were greatly reduced to  $1.9 \pm 1.7\%$  from control values of  $34.4 \pm 1.8\%$  and the percentage moisture of the blood of dying fish increased from  $84.5 \pm 1.0$  to  $96.4 \pm 1.5\%$ .

Because most of the sea lamprey's diet consists of blood, and teleosts have a relatively small blood volume, blood loss can result in marked changes in various blood features. By monitoring various blood features during the attack period and during wound healing one can determine what changes take place during lamprey attachment and how the host recovers during wound healing. Because the hemopoietic centers of the kidney and spleen will be taxed during lamprey feeding, the pathology of these organs was studied to determine what effects, if any, occur.

## METHODS AND MATERIALS

### EXPERIMENTAL ANIMALS

Rainbow trout used in the study were obtained from the Midwest Trout Farm, Harrison, Michigan. Before experimentation the fish were held in 1,000 L circular tanks that received flowing 12°C well water of pH 7.1, hardness 330 mg CaCO<sub>3</sub>/L and alkalinity 325 mg CaCO<sub>3</sub>/L. Dissolved oxygen was 9.0 mg/L or higher. The fish were fed Trout Chow (Ralston Purina, Checkerboard Square, St. Louis, MO) *ad libitum*. The averages for total length and weight of the trout used in the wound development study were 30.6 cm and 309.1 g; the averages for those in the wound healing group were 31.1 cm and 328.4 g, respectively.

Sea lamprey, obtained from the U.S. Fish and Wildlife Service Laboratory at Hammond Bay, Michigan, were juveniles that had recently completed metamorphosis. Lamprey were maintained in 150 L tanks receiving flowing well water from the same source as that used for the trout. The lamprey were allowed to feed on carp (*Cyprinus carpio*) before experimentation. All lamprey were actively growing, therefore those used in the later wound healing study were larger on the average (22.9 cm and 25.3 g) than those of the wound development study (19.1 cm and 13.5 g).

### EXPERIMENTAL PROCEDURE FOR WOUND DEVELOPMENT

Individual trout were weighed, measured (total length), and placed singly in a 150 L fiberglass tank containing sea lamprey. When one of the lampreys attached itself to the trout, the time and position of attachment were noted and the remaining lampreys were removed to a holding tank. The lamprey was allowed to feed on the trout for a prescribed period after which the lamprey and trout were anesthetized in 80 mg/L tricaine methane sulfonate (MS-222), separated, weighed, and measured. Blood samples and the hemopoietic tissues of the kidney and spleen were collected from wounded fish at 4 h, 12 h, 2 days, and 10 days after the initial lamprey attachment. Five fish were used for each sampling period.

### HEMATOLOGY PROCEDURE

A 0.5 cc blood sample was collected from the caudal blood vessel of the trout with a 22 gauge needle and 3.0 cc syringe. The blood was then placed in a 3.0 cc vacutainer tube treated with EDTA to prevent clotting.

Two blood smears were prepared immediately. One smear was stained with Wright's stain and the other was fixed in absolute methanol as a spare. The stained smear was examined under oil immersion (1,000x) for immature red and white blood cell differential counts. The immature red blood cells (RBCs) were determined as a percentage of the first 500 red blood cells counted. The white blood cell count included the differentiation of the first 100 white blood cells into lymphocytes, thrombocytes, and granulocytic, metagranulocytic, immature, or segmented neutrophils (Lehmann and Sturenberg 1975).

Hematocrit and hemoglobin were determined by the microhematocrit and cyanmethemoglobin methods, respectively. Two heparinized microhematocrit capillary tubes were used in each hematocrit test and the two readings were averaged.

Leucocrit was determined by measuring the buffy coat at the surface of the packed red blood cells using an ocular micrometer and the determination was made by techniques described by McLeay and Gordon (1977).

#### HISTOPATHOLOGIC TECHNIQUE

Tissue samples of the spleen and anterior kidney were removed and fixed immediately in 10% buffered neutral formalin, then imbedded in paraffin, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin (Luna 1960).

#### EXPERIMENTAL PROCEDURE FOR WOUND HEALING

Lamprey were allowed to attach on trout for 8 days. The fish were then separated from the lamprey by anesthesia in MS-222 and returned to holding tanks for a prescribed time period. To monitor the hosts' recovery, blood samples for hematocrit, hemoglobin, leucocrit, and blood smears were taken immediately after lamprey detachment and at 1 week intervals thereafter. The trout were weighed and measured at the time of each blood collection. Five wounded fish were sacrificed to obtain tissue samples of the spleen and anterior kidney at 2 days, 2 weeks, 1 month, and 3 months after lamprey detachment.

## RESULTS AND DISCUSSION

#### BLOOD FEATURES OF WOUND DEVELOPING AND WOUND HEALING FISH

During lamprey attachment the hematocrit progressively increased from a control value of  $24.1 \pm 1.8\%$  to a significantly higher value of  $30.7 \pm 1.8\%$  after 12 h of attachment (Fig. 1A). This significant rise in the hematocrit could be attributed in part to stress response (Casillas and Smith 1977). Wedemeyer (1970), Nilsson and Grove (1974), and Schreck et al. (1976) found the stress response in fish resulted in additional erythrocytes entering the circulatory system. The hematocrit was  $27.9 \pm 1.4\%$  at 10 days of lamprey attachment indicating the inability of the smaller lamprey (19.1 cm and 13.5 g) to induce anemia even after 10 days of attack.

Changes in the hemoglobin concentration closely paralleled the changes in the hematocrit (Fig. 1C). The initial hemoglobin value was  $6.6 \pm 0.7$  g/dl but after 12 h of lamprey attachment it was  $8.6 \pm 0.4$  g/dl. This significant rise was consistent with the rise in the hematocrit. The hemoglobin then decreased to  $7.8 \pm 0.4$  g/dl after 10 days of lamprey attachment, following in line with the hematocrit. There was no significant change in the red blood cell (RBC) precursors during this wound development period (Fig. 1E).

The fish in the wound healing group all experienced an 8 day sea lamprey

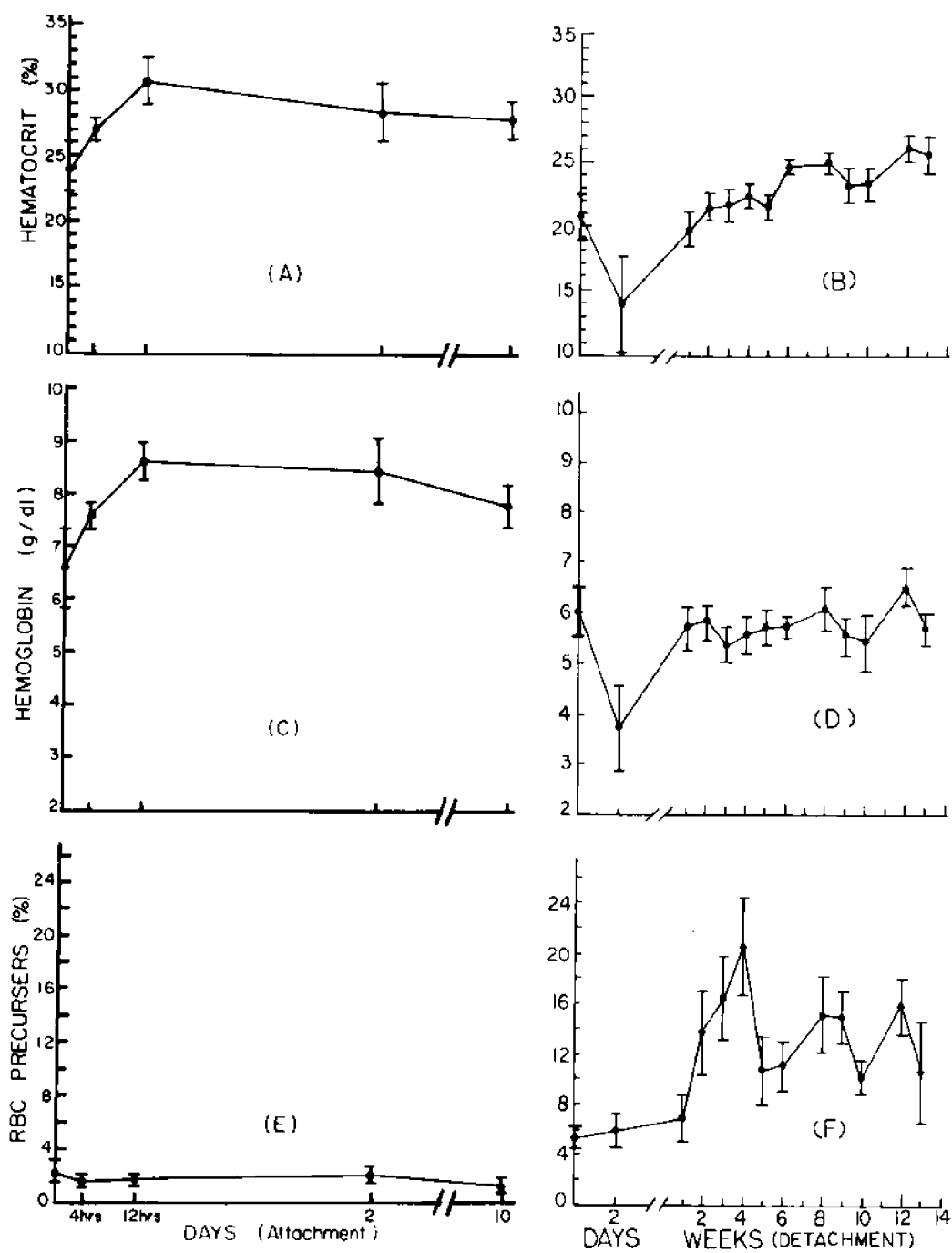


Fig. 1. Hematocrit, hemoglobin, and red blood cell precursor values during wound development and wound healing in lamprey-attacked fish.

- (A) Hematocrit (%) in wound development fish.
- (B) Hematocrit (%) in wound healing fish.
- (C) Hemoglobin (g/dl) in wound development fish.
- (D) Hemoglobin (g/dl) in wound healing fish.
- (E) Red blood cell precursors (%) in wound development fish.
- (F) Red blood cell precursors (%) in wound healing fish.



attachment. The hematocrit curve was similar to that of the hemoglobin during the wound healing period (Fig. 1B and D). Hematocrit and hemoglobin at lamprey detachment were  $20.7 \pm 1.9\%$  and  $6.0 \pm 0.5$  g/dl, respectively, and both dropped significantly to  $14.0 \pm 3.5\%$  and  $3.7 \pm 0.9$  g/dl at 2 days post detachment. The decline was attributed to a dilution effect on the blood as the wound was open and the fish was no doubt subjected to a flux of incoming water in the wound area. Kirk (1974) reported that the osmoregulatory status of fish may cause the hematocrit to change. The hematocrit and hemoglobin increased to  $19.8 \pm 1.3\%$  and  $5.7 \pm 0.4$  g/dl, respectively, at 1 week. The hematocrit continued to rise slightly over time, with some slight fluctuations, reaching a value of  $25.7 \pm 1.4\%$  at 3 months; the hemoglobin continued at a fluctuating plateau to 3 months when it had a value of  $5.7 \pm 0.2$  g/dl.

The increased hematocrit during wound healing was attributed to the formation of new epidermis over the wound area which would prevent hemodilution due to the influx of water. An accompanying red blood cell precursor response could also account for the rising hematocrit at 1 week and beyond (Fig. 1F). The red blood cell precursor value at detachment was  $5.3 \pm 0.9\%$  of the total red blood cells and was significantly higher than the control value of  $2.2 \pm 0.7\%$ . The number of RBC precursors continued to rise significantly after lamprey detachment. By week 2 the value was  $13.9 \pm 3.1\%$  and by week 4 the value reached a peak of  $20.9 \pm 3.5\%$ . By week 5 the number of RBC precursors had dropped to  $10.6 \pm 3.0\%$  and remained at a highly fluctuating level to 3 months when the value was  $10.4 \pm 4.2\%$ . One would expect that the increased number of RBC precursors during wound healing would result in a lower hemoglobin concentration because of the reduced amount of hemoglobin carried by these immature cells (Walker 1975). Although the hemoglobin concentration fluctuated around 5.7 g/dl from 1 week through 3 months of wound healing, it was lower than the hemoglobin concentration of 6.6 g/dl in fish that did not experience a lamprey attack (Fig. 1C and D).

The RBC precursor response tends to agree with results reported by Walker (1972). After withdrawing 20 to 30% of the whole blood volume from rainbow trout he found the reticulocytes appeared 5 days later and a high count of 40.4% remained at 20 days. He concluded the reticulocyte increase in the peripheral circulation is long-term since the response was still in progress on day 20.

Walker (1975) removed 40% of the blood volume from rainbow trout within a 7 day period and reported significant effects on the hematocrit, hemoglobin, and reticulocyte count. He found significant hematocrit recovery after 16 days and the hemoglobin concentration had recovered 30 days after bleeding. This difference in hematocrit and hemoglobin recovery indicated a replacement of the lost red blood cells by immature cells which did not contain complete hemoglobin molecules.

McLeay and Gordon (1977) introduced a test called the leucocrit which is the volume of packed leucocytes and thrombocytes expressed as a percentage of the whole blood. They found that the number of circulating leucocytes and thrombocytes was a more accurate reflection of a fish's reaction to stress than the number of erythrocytes. Using coho salmon (*Oncorhynchus kisutch*) and rainbow trout, they observed that the leucocrit and leucocyte-thrombocyte counts for

both species were depressed from control values after 96 h of exposure to stressful conditions.

During wound development in our study the leucocrit value decreased slightly, but not significantly, from the control value of  $0.97 \pm 0.10\%$  to  $0.89 \pm 0.04\%$  and  $0.84 \pm 0.13\%$  at 12 h and 10 day attachments, respectively (Fig. 2A). The differential leucocyte count indicated that the lymphocytes decreased during lamprey attachment. The lymphocyte control value of  $95.6 \pm 1.3\%$  dropped significantly to  $91.4 \pm 1.0\%$  and  $79.4 \pm 6.1\%$  at the 4 and 12 h attachment periods, respectively, before rising to  $82.2 \pm 7.5\%$  at the 2 day lamprey attachment period (Fig. 2C). There were some relative increases in the neutrophilic series and thrombocyte percentages during wound development which may have balanced out the decreased percentage of lymphocytes, thus explaining why the leucocrit did not drop significantly during this time. Many researchers have shown that stress in teleosts plays an important role in lymphopenia accompanied by neutrophilia (Weinreb 1958; Slicher 1961; Belova 1965; McLeay 1973a, 1973c; Bennett and Gaudio Neville 1975).

Wistar and Hildermann (1960) found that ACTH and adrenocorticoids cause a depression of lymphoid cells and that chronic stress results in leucopenia and loss of immunological responsiveness in mammals. There is also evidence that stress causes increases in circulating adrenal corticosteroid levels in teleostean fishes (Hane et al. 1966; Fagerlund 1967; Wedemeyer 1969; Singley and Chavin 1975a, 1975b; Mazeaud et al. 1977). Non-specific stress in fish results in lymphopenia (Weinreb 1958; Ball and Slicher 1962; Pickford et al. 1971; McLeay 1973a, 1973b, 1973c, 1975; Bennett and Gaudio Neville 1975).

In the wound healing group of our experiment, the leucocrit at detachment was  $0.59 \pm 0.04\%$  which was significantly lower than the control leucocrit of  $0.97 \pm 0.10\%$  (Fig. 2B). After detachment the leucocrit increased significantly to a value of  $1.16 \pm 0.09\%$  at 1 week and remained at a fluctuating plateau for nearly 3 months. The decreased leucocrit at detachment would tend to be caused by a decreased lymphocyte number. The lymphocytes at detachment had a value of  $92.8 \pm 2.2\%$  (Fig. 2D). Although a relatively high lymphocyte percentage was present, this only represents a relative percentage of the total white blood cells. In fact, there probably was an absolute drop in both lymphocytes and the neutrophilic series as evidenced by the decreased leucocrit. The relative lymphocyte percentage then dropped significantly to  $62.7 \pm 8.1\%$  at 2 days before increasing again to a sustained higher level at 1 week. The lower leucocrit and lymphocyte percentages at 2 days could be attributed to the stress caused by the possible influx of water through the opened wound area. The lymphopenia at 2 days was also accompanied by an increase in the percentage of the neutrophilic series.

During wound development the thrombocyte concentration increased significantly from a control value of  $0.8 \pm 0.5\%$  to  $10.4 \pm 2.2\%$  at the 12 h attachment (Fig. 2E). This value then fell somewhat at 2 days attachment. This conforms with the findings of Casillas and Smith (1977) who found that thrombocyte counts increase after stress. The thrombocyte concentration during wound healing remained at a slightly elevated level throughout the 3 month period (Fig. 2F).

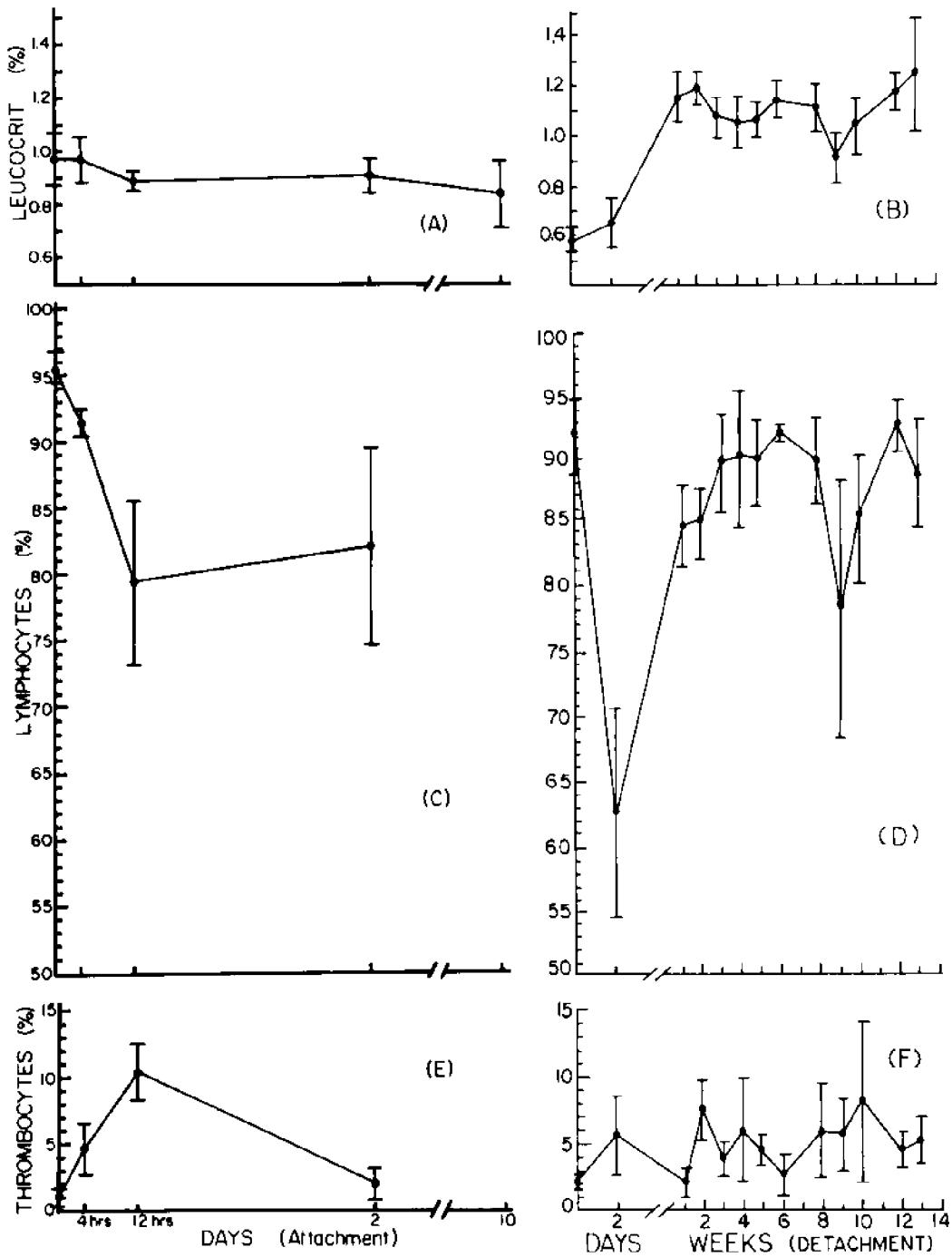


FIG. 2. Leucocrit, lymphocyte, and thrombocyte values during wound development and wound healing in lamprey-attacked fish. The blood smears obtained at 10 day lamprey attachment were of poor quality, therefore a white blood cell differential was not obtained.

- (A) Leucocrit (%) in wound development fish.
- (B) Leucocrit (%) in wound healing fish.
- (C) Lymphocyte percent of white blood cell differential in wound development fish.
- (D) Lymphocyte percent of white blood cell differential in wound healing fish.
- (E) Thrombocyte percent of white blood cell differential in wound development fish.
- (F) Thrombocyte percent of white blood cell differential in wound healing fish.

During wound development the neutrophilic series increased from a control value of  $3.6 \pm 1.8\%$  to  $15.8 \pm 6.3\%$  after 2 days of attachment (Fig. 3A). This indicates the start of the inflammatory response of the fish toward the wound.

In wound healing, the neutrophilic series at detachment was  $4.7 \pm 1.9\%$  and then rose sharply to  $31.5 \pm 7.2\%$  at 2 days (Fig. 3B). This also happened to be the time when the highest cellular response was noted in histological sections of the wound area. The increased percentage of the neutrophilic series in the blood at this time was most likely a protective mechanism as the wound was open to the environment and was an ideal entry area for pathogens. The neutrophilic series remained elevated at week 1 and had a value of  $13.4 \pm 2.3\%$ .

During wound development the granulocytes and immature and segmented neutrophils increased through the attachment periods; the immature and segmented neutrophils had the largest increases at 2 days attachment (Fig. 3 C, E and G). The metagranulocytes were present in insignificant numbers.

During wound healing the granulocytes and immature and segmented neutrophils were significantly elevated at 2 days; the segmented neutrophils attained the highest percentage of  $20.3 \pm 4.7\%$  (Fig. 3 D, F, and H). This demonstrates the ability of the fish to respond to adverse conditions by increasing the relative percentage of mature neutrophils which would no doubt be the most capable of handling incoming pathogens. Metagranulocytes were again insignificant during wound healing.

No cells were identified as monocytes, basophils, or eosinophils in our study. Ellis (1977) reported that the literature is extremely confused on the designation of monocytes in fishes and some workers even deny the existence of monocytes in teleost fish. McCarthy et al. (1973) were unable to find any cells in the blood of rainbow trout that resembled the mammalian monocytes. Catton (1951) had similar difficulty with the blood cells of trout and roach. Blaxhall and Daisley (1973) could not identify monocytes in the blood of brown trout (*Salmo trutta*) though they reported that neutrophils and metamyelocytes could easily be mistaken unless cytochemical staining methods were used. Basophils and eosinophils have not been observed from the blood of rainbow trout (Klontz 1972) and brown trout (Blaxhall and Daisley 1973). Ellis (1977) claimed that the entire literature concerning eosinophils in fish is contradictory in that there have been reports of their presence and absence in many fish species. Ellis (1976) reported the absence of eosinophils and basophils from the circulation in plaice (*Pleuronectes platessa*).

#### BLOOD FEATURES OF MORTALLY WOUNDED FISH

The hemoglobin, hematocrit and leucocrit values of mortally wounded fish at detachment were as low as 0.2 g/dl, 2.0% and 0.30%, respectively (Table 1). The red blood cell precursors were as high as 15% in one fish.

White blood cell differential counts from a fish near death 10 days after lamprey detachment had a neutrophilic series of 0% at detachment, 4.0% at 1 week, and 25.0% at 10 days after detachment. The immature and segmented neutrophils made up the greatest percentage of the neutrophilic series. The lymphocytes were 100% at detachment, 96.0% at 1 week, and 74.0% at 10 days

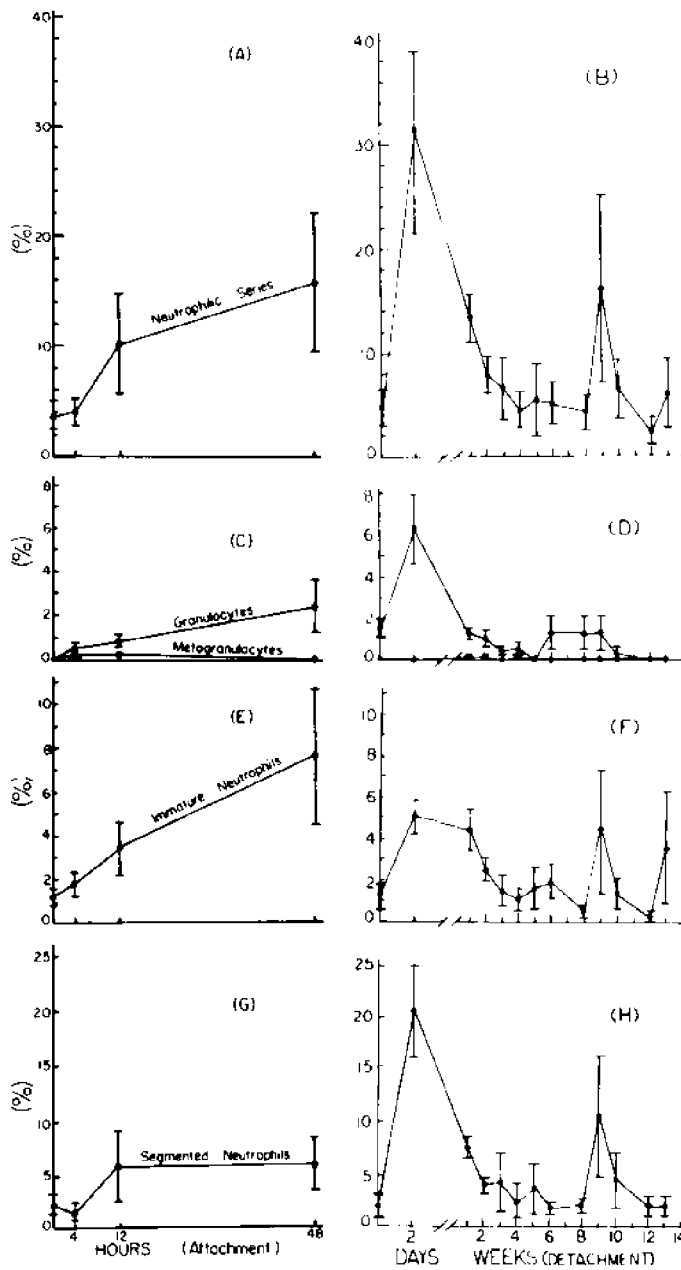


FIG. 3. Neutrophilic series and constituents during wound development and wound healing in lamprey-attacked fish expressed as a percentage of the white blood cell differential. The blood smears obtained at 10 day lamprey attachment were of poor quality therefore a white blood cell differential was not obtained.

- (A) Neutrophilic series (%) in wound development fish.
- (B) Neutrophilic series (%) in wound healing fish.
- (C) Granulocyte (%) and metagranulocyte (%) in wound development fish.
- (D) Granulocyte (%) and metagranulocyte (%) in wound healing fish.
- (E) Immature neutrophil (%) in wound development fish.
- (F) Immature neutrophil (%) in wound healing fish.
- (G) Segmented neutrophil (%) in wound development fish.
- (H) Segmented neutrophil (%) in wound healing fish.

after detachment. The thrombocytes attained a value of 1.0% 10 days following detachment.

It is obvious that the large blood loss from the fish plays a role in its death. Using the formula  $\log y = 3.311 - 1.533 \log x$  which was developed by Farmer et al. (1975), it is possible to calculate x which is an estimate of the amount of blood lost per day, expressed as a percentage of the host fishes blood volume, since one knows y, the time to its death in days. For instance, if the fish near death after 5 days of lamprey attachment (Table 1) was used to find the daily blood volume loss, a value of about 50% would be obtained. This is indicative of the great demands on the hemopoietic organs of the fish, because an amount equivalent to its blood volume would have to be replaced every 2 days. Because the RBC precursors were only 15% of the red blood cells (Table 1), it is obvious that once the blood reserves from the spleen and kidney are depleted the fish would die.

One fish near death 10 days following lamprey detachment had progressively lost weight from 311.5 g at detachment to 279.9 g 10 days later. The wound area became infected and fungal infection was seen at 4 days. The hematocrit dropped from 15% at lamprey detachment to 10% at 7 and 10 days following detachment (Table 1). This drop could be attributed to extensive hemorrhaging that occurred in the wound area during this time. The number of RBC precursors had risen during this period to 9.6% at 10 days. The leucocrit was depressed to a level of 0.44% at detachment indicating its immunological response was somewhat depressed; the white blood cell differential showed 0% neutrophilic series and 100% lymphocytes. Ten days following lamprey detachment, the neutrophilic series increased to 25% of the total white blood cells of which most were immature and segmented neurophils, and the lymphocytes dropped to 74%. The leucocrit at this time also rose to 1.12% indicating an absolute rise in the neutrophils with the lymphocytes staying depressed possibly because of the increased stress condition at this time. This increase in neutrophils could be attributed to the infected wound. Similar results were reported by Hines and Spira (1973) who found that mirror carp infected with *Ichthyophthirius*

TABLE 1. Blood features of mortally wounded fish

Length of lamprey attachment	Hematocrit at detachment (%)	Hemoglobin at detachment (g/dl)	Leucocrit at detachment (%)	RBC Precursors at detachment (%)
5 days	8.0	1.7	0.44	15.0
7 days	2.0	0.2	0.30	not obtained
8 days	2.0	0.4	0.59	not obtained
8 days*	15.0	6.0	0.44	0.4
1 week after detachment	10.0	3.4	0.59	1.5
10 days after detachment	10.0	5.0	1.12	9.6

\*This fish was not near death until 10 days following detachment and thus blood samples were taken at 1 week and 10 days.

*multifiliis* had a sharp lymphocyte drop with a concurrent rise in neutrophil percentages.

Lennon (1954) observed that rainbow trout mortally wounded by sea lamprey had erythrocyte counts which were 14.9% of control fish and their blood hemoglobin was reduced by at least 90%. There was a 49.2% drop in white cells in lamprey wounded trout compared with healthy rainbow trout. This is consistent with our research results.

#### HISTOPATHOLOGY OF THE KIDNEY AND SPLEEN

The most striking changes occurred in the spleen (Fig. 4). Some fish exhibited blood congestion in the red pulp regions of the spleen especially those of the 10 day wound development and 2 day wound healing groups (Fig. 5). The lymphoid (white pulp) region may have released excess numbers of red blood cells into the red pulp region to meet the demand of lamprey feeding. The most striking change in the spleen was the depletion of the white pulp region in the anemic fish with a subsequent loss of the once discernible red and white pulp regions (Fig. 6). This is evidence of a degeneration of hemopoietic tissue due to the excess burden of blood loss. No significant changes in the hemopoietic tissues in the kidneys of any of the fish were evident.

Stress may also play a role in the white pulp depletion in the spleen. Rasquin (1951) reported the spleen was completely depleted of lymphoid tissue in *Astyanax mexicanus* in response to an injection of ACTH, or when the fish were held under adverse conditions.

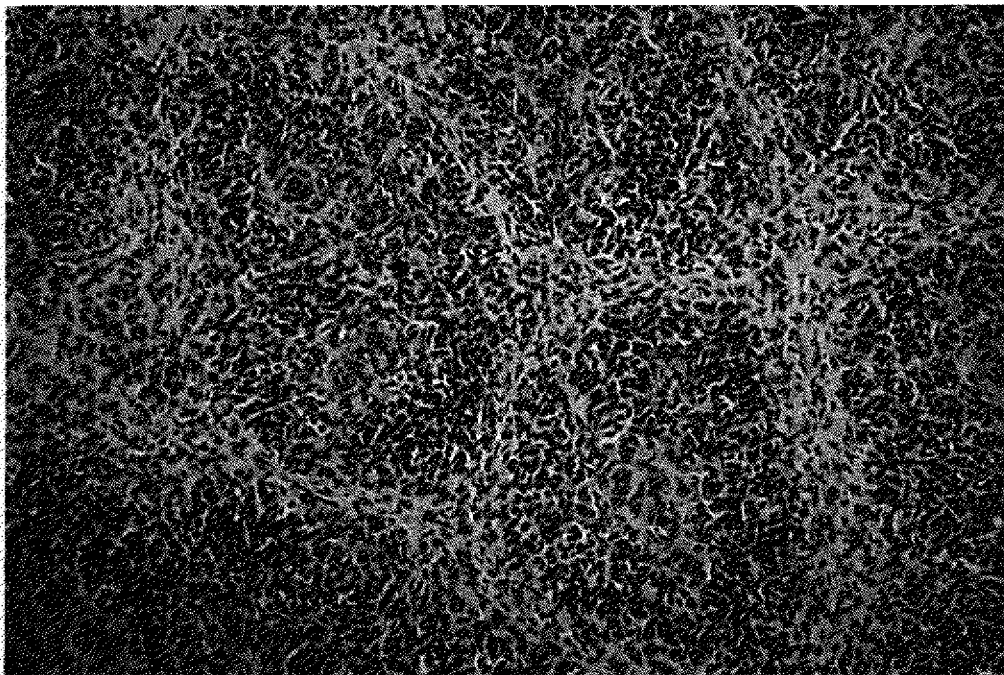


FIG. 4. A normal rainbow trout spleen showing the red and white pulp regions H & E X100.

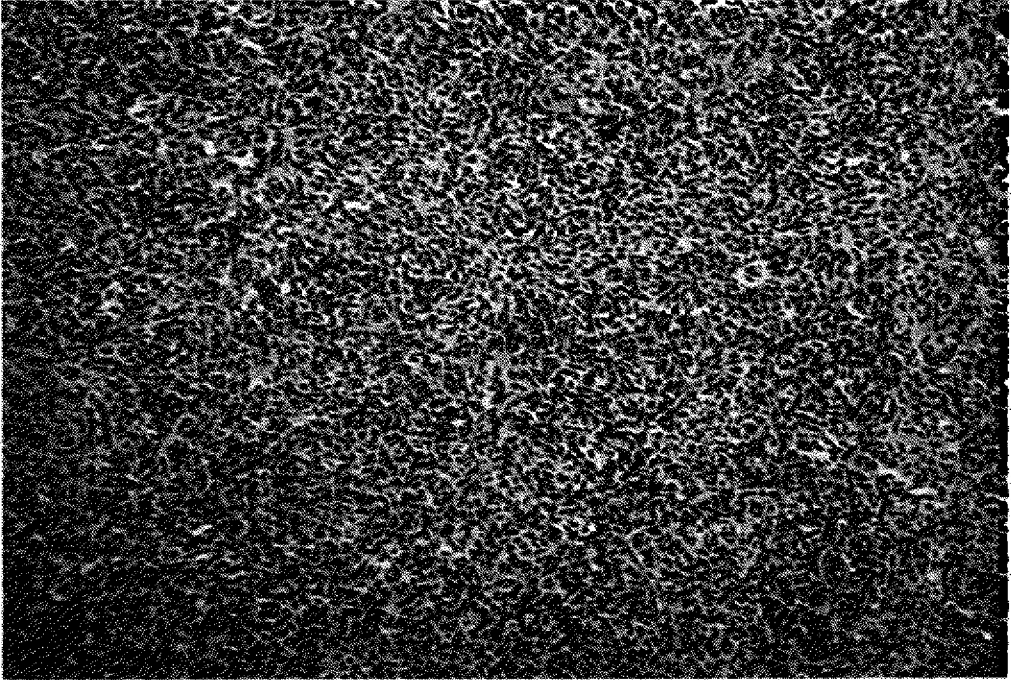


FIG. 5. Red blood cell congestion in the red pulp region of the spleen. H & E. X100.

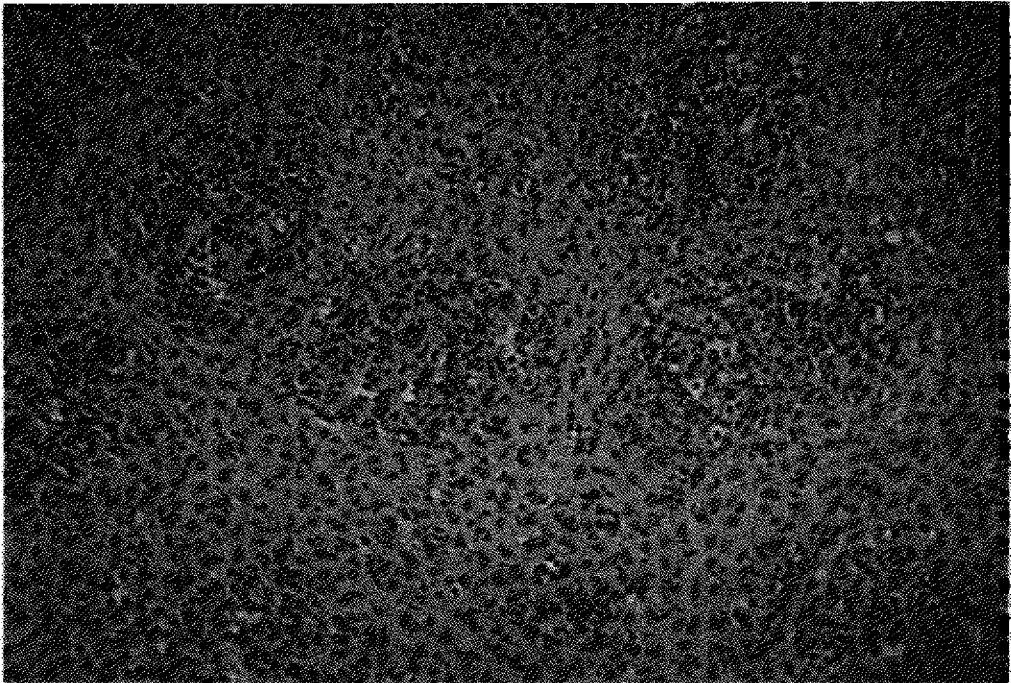


FIG. 6. Spleen from a rainbow trout that was near death after five days of sea lamprey attack—note the diminished white pulp regions. H & E. X100.



APPENDIX

APPENDIX TABLE 1. Hematocrit, hemoglobin, red blood cell precursor, and leucocrit values for wound development and wound healing fish.  $\bar{X} \pm S.E.$

Wound stage and time	Hematocrit (%)	Hemoglobin (g/dl)	RBC precursors (%)	Leucocrit (%)
<b>Wound development</b>				
Control	24.1 ± 1.8	6.6 ± 0.7	2.2 ± 0.7	0.97 ± 0.10
4 h	27.0 ± 0.8	7.6 ± 0.3	1.5 ± 0.4	0.97 ± 0.09
12 h	30.7 ± 1.8	8.6 ± 0.4	1.5 ± 0.4	0.89 ± 0.04
2 days	28.4 ± 2.3	8.5 ± 0.6	1.9 ± 0.6	0.91 ± 0.07
10 days	27.9 ± 1.4	7.8 ± 0.4	1.1 ± 0.4	0.84 ± 0.13
<b>Wound healing</b>				
<b>Detachment</b>				
2 days	20.7 ± 1.9	6.0 ± 0.5	5.3 ± 0.9	0.59 ± 0.04
1 week	14.0 ± 3.5	3.7 ± 0.9	6.0 ± 1.1	0.66 ± 0.09
2 weeks	19.8 ± 1.3	5.7 ± 0.4	6.9 ± 1.9	1.16 ± 0.09
3 weeks	21.5 ± 1.1	5.8 ± 0.4	13.9 ± 3.1	1.19 ± 0.06
4 weeks	21.6 ± 1.3	5.4 ± 0.3	16.3 ± 3.6	1.07 ± 0.08
5 weeks	22.4 ± 0.9	5.6 ± 0.3	20.9 ± 3.5	1.05 ± 0.10
6 weeks	21.6 ± 0.9	5.7 ± 0.4	10.6 ± 3.0	1.06 ± 0.07
8 weeks	24.7 ± 0.4	5.7 ± 0.2	10.9 ± 2.3	1.14 ± 0.07
9 weeks	25.0 ± 0.7	6.1 ± 0.4	15.4 ± 2.9	1.11 ± 0.09
10 weeks	23.3 ± 1.3	5.5 ± 0.3	15.2 ± 1.7	0.91 ± 0.10
12 weeks	23.4 ± 1.2	5.4 ± 0.5	10.1 ± 1.4	1.04 ± 0.11
13 weeks	26.1 ± 0.9	6.5 ± 0.3	15.9 ± 2.3	1.17 ± 0.07
13 weeks	25.7 ± 1.4	5.7 ± 0.2	10.4 ± 4.2	1.25 ± 0.22

APPENDIX TABLE 2. Components of the white blood cell differential by percent occurrence for wound development and wound healing fish.  $\bar{X} \pm S.E.$

Wound stage and time	Neutrophilic				Metagranulocytes	Immature Neutrophils	Segmented Neutrophils	Lymphocytes	Thrombocytes
	Series	Granulocytes							
<b>Wound development</b>									
Control	3.6±1.2	0	0	1.2±0.4	2.4±0.9	95.6±1.3	0.8±0.5		
4 h	4.0±1.4	0.4±0.4	0.2±0.2	1.8±0.6	1.6±0.9	91.4±1.0	4.6±2.0		
12 h	10.2±4.5	0.8±0.4	0.2±0.2	3.4±1.2	5.8±3.3	79.4±6.2	10.4±2.2		
2 days	15.8±6.3	2.4±1.3	0	7.6±3.1	5.8±2.6	82.2±7.5	2.0±1.2		
<b>Wound healing</b>									
Detachment	4.7±1.9	1.5±0.5	0	1.2±0.7	2.0±1.1	92.8±2.2	2.2±0.7		
2 days	31.5±7.2	6.3±1.7	0	5.0±0.9	20.3±4.7	62.7±8.1	5.7±2.8		
1 week	13.4±2.3	1.3±0.3	0.1±0.1	4.4±1.0	7.7±1.5	84.5±3.2	2.1±1.1		
2 weeks	7.9±1.4	1.0±0.4	0.1±0.1	2.6±0.5	4.1±0.8	84.7±2.8	7.5±2.2		
3 weeks	6.5±3.3	0.4±0.2	0	1.6±0.7	4.5±2.6	89.6±4.1	3.9±1.3		
4 weeks	4.3±1.9	0.5±0.3	0.1±0.1	1.1±0.5	2.5±1.4	89.9±5.6	5.9±3.9		
5 weeks	5.5±3.2	0	0	1.7±0.9	3.8±2.5	89.7±3.5	4.7±1.2		
6 weeks	5.3±1.9	1.3±0.8	0	2.0±0.8	2.0±0.8	92.0±0.4	2.7±1.6		
8 weeks	4.3±1.6	1.3±0.8	0	0.5±0.3	2.5±0.9	89.8±3.5	6.0±3.5		
9 weeks	16.0±9.1	1.3±0.9	0	4.5±2.9	10.3±5.6	78.3±10.0	5.7±2.7		
10 weeks	6.5±2.8	0.3±0.3	0	1.5±0.7	4.8±2.1	85.3±5.1	8.3±5.7		
12 weeks	2.5±1.2	0	0	0.3±0.3	2.3±0.9	92.8±2.1	4.7±1.3		
13 weeks	6.0±3.5	0	0	3.7±2.7	2.3±0.9	88.7±4.5	5.3±1.7		

## REFERENCES

- BALL, J. N. and A. M. SLICHER.  
1962. Influence of hypophysectomy and adrenocortical inhibitor SU-4885 on the stress response of the white blood cells in the teleost, *Molliensia lutipinna*. Nature, London. 196:1331-1332.
- BELOVA, A. V.  
1965. The effect of transportation conditions on the composition of blood in the young humpback salmon grown in fish hatcheries of Murman. Dokl. Akad. Nauk. SSSR 161:466-468.
- BENNETT, M. F. and C. GAUDIO NEVILLE.  
1975. Effects of cold shock on the distribution of leucocytes in goldfish *Carassius auratus*. J. Comp. Physiol. 98:213-216.
- BLAXHALL, P. C. and K. W. DAISLEY.  
1973. Routine hematological methods for use with fish blood. J. Fish. Biol. 5:771-781.
- CASILLAS E. and L. S. SMITH.  
1977. Effect of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). J. Fish. Biol. 10:481-491.
- CATTON, W. T.  
1951. Blood cell formation in certain teleost fishes. Blood. J. Hematology 6:39-60.
- ELLIS, A. E.  
1976. Leucocytes and related cells in the plaice *Pleuronectes platessa*. J. Fish. Biol. 8:143-156.  
1977. The leucocytes of fish: A review J. Fish. Biol. 11:453-491.
- FAGERLUND, U. H. M.  
1967. Plasma cortisol concentration in relation to stress in adult sockeye salmon during the freshwater stage of their life cycle. Gen. Comp. Endocrinol. 8:197-207.
- FARMER, G. J., F. W. H. BEAMISH and G. A. ROBINSON.  
1975. Food consumption of the adult landlocked sea lamprey, *Petromyzon marinus*, L. Comp. Biochem. Physiol. 50:753-757.
- HANE, S., O. H. ROBERTSON, B. C. WEXLER and M. A. KRUPP.  
1966. Adrenocortical response to stress and ACTH in Pacific salmon (*Onchorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*) at successive stages in the sexual cycle. Endocrinol. 78:791-800.
- HINES, R. and D. T. SPIRA.  
1973. Ichthyophthiriasis in the mirror carp. III. Leukocyte response. J. Fish. Biol. 5:527-534.
- KIRK, W. L.  
1974. The effects of hypoxia on certain blood and tissue electrolytes of channel catfish, *Ictalurus punctatus* (Rafinesque). Trans. Am. Fish Soc. 103:593-600.
- KLONTZ, G. W.  
1972. Haematological techniques and immune response in rainbow trout. In: Diseases of fish (Ed. Mawdesley-Thomas, L.E.), Symp. Zool. Soc. Lond. No. 30. New York and London: Academic Press. pp. 89-99.

- LEHMANN, J. and F. J. STURENBERG.  
 1975. Beschreibung und Darstellung der wichtigsten zellen in der Blutbildungs-  
 stätte und im peripheren Blugefässsystem. Gewässer und Abwasser No.  
 55/56, 123 p.
- LENNON, R. E.  
 1954. Feeding mechanism of the sea lamprey and its effect on host fishes. U.S.  
 Dept. of Int., Fish Wildl. Serv., Fish Bull., 98.
- LUNA, L. G. ed.  
 1960. Manual of histologic staining methods of the armed forces institute of  
 pathology. 3rd ed. McGraw Hill, New York.
- MAZEAUD, M., F. MAZEAUD and E. DONALDSON.  
 1977. Primary and secondary effects of stress in fish: Some new data with a general  
 review. Trans. Am. Fish. Soc. 106:201-212.
- MCCARTHY, D. H., J. P. STEVENSON and M. S. ROBERTS.  
 1973. Some blood parameters of rainbow trout (*Salmo gairdneri*). J. Fish. Biol.  
 5:1-8.
- MCLEAY, D. J.  
 1973a. Effects of ACTH on the pituitary-interrenal axis and abundance of white  
 blood cell types in juvenile coho salmon, *Oncorhynchus kisutch*. Gen.  
 Comp. Endocrinol. 21:431-440.  
 1973b. Effects of cortisol and dexamethasone on the pituitary-interrenal axis and  
 abundance of white blood cell types in juvenile coho salmon, *Oncorhynchus*  
*kisutch*. Gen. Comp. Endocrinol. 21:441-450.  
 1973c. Effects of a 12-hr and 25-day exposure to kraft pulpmill effluent on the blood  
 and tissues of juvenile coho salmon (*Oncorhynchus kisutch*). J. Fish. Res.  
 Board Can. 30:395-400.  
 1975. Variations in the pituitary-interrenal axis and the abundance of circulating  
 blood-cell types in juvenile coho salmon, *Oncorhynchus kisutch*, during  
 stream residence. Can. J. Zool. 53:1882-1891.
- MCLEAY, D. J. and M. R. GORDON.  
 1977. Leucocrit: a simple hematological technique for measuring acute stress in  
 salmonid fish, including stressful concentrations of pulpmill effluent. J.  
 Fish. Res. Board Can. 34:2164-2175.
- NILSSON, S. and D. J. GROVE.  
 1974. Adrenergic and cholinergic innervation of the spleen of the cod: *Gadus*  
*morhua*. European J. Pharmacol. 28:135-143.
- PICKFORD, G. E., A. K. STRAVASTAVIA, A. M. SLICHER and P. K. T. PANG.  
 1971. The stress response in the abundance of circulatory leucocytes in the killfish,  
*Fundulus heteroclitus*. I. The cold-shock sequence and the effects of  
 hypophysectomy. J. Exp. Zool. 177:89-96.
- RASQUIN, P.  
 1951. Effects of carp pituitary and mammalian ACTH on the endocrine and  
 lymphoid systems of the teleost, *Astyanax mexicanus*, J. Exp. Zool.  
 117:317-358.
- SCHRECK, C. B., R. A. WHALEY, M. L. BASS, O. E. MAUGHAN, and M.  
 SOLAZZI.  
 1976. Physiological responses of rainbow trout (*Salmo gairdneri*) to electroshock.  
 J. Fish. Res. Board Can. 33:76-84.
- SINGLEY, J. A., and W. CHAVIN.  
 1975a. Serum cortisol in normal goldfish (*Carassius auratus* L.) Comp. Biochem.  
 Physiol. 50A:77-82.

- 1975b. The adrenocortical-hypophyseal response to saline stress in the goldfish, *Carassius auratus* L. *Comp. Biochem. Physiol.* 51A:749-756.
- SLICHER, A. M.  
1961. Endocrinological and haematological studies in *Fundulus heteroclitus* (Linn.) *Bull. Bingham oceanogr. coll.* 17:3-55.
- WALKER, R. L.  
1972. In vitro study of erythropoieses in rainbow trout (*Salmo gairdneri*) using  $^{59}\text{FeCl}_3$ . M.S. Thesis. Michigan State University. 100 pp.  
1975. Uptake, distribution, and incorporation of  $^{59}\text{Fe}$  in tissue and blood of rainbow trout (*Salmo gairdneri*) Ph.D. Dissertation. Michigan State University. 118 pp.
- WEDEMEYER, G. A.  
1969. Stress-induced ascorbid acid depletion and cortisol production in two salmonid fishes. *Comp. Biochem. Physiol.* 29:1247-1251.  
1970. The role of stress in the disease resistance of fishes. In *A Symposium on Diseases of Fishes and Shellfishes* (S. F. Snieszko, ed.), Washington, D.C.: American Fisheries Soc. 30-35.
- WEINREB, E. L.  
1958. Studies on the histology and histopathology of the rainbow trout, *Salmo gairdneri irideus*. I. Haematology under normal and experimental conditions of inflammation. *Zoologica N.Y.* 43:145-154.
- WISTAR, R. and HILDERMANN.  
1960. Effect of stress on skin transplantation immunity in mice. *Science N.Y.* 131:159-160.

NATIONAL SEA GRANT DEPOSITORY  
 PELL LIBRARY BUILDING  
 URI, NARRAGANSETT BAY CAMPUS  
 NARRAGANSETT, RI 02882  
**GREAT LAKES FISHERY COMMISSION**  
**TECHNICAL REPORT SERIES**

- No. 1. Use of 3-trifluoromethyl-4-nitrophenol as a selective sea lamprey larvicide, by *Vernon C. Applegate, John H. Howell, James W. Moffett, B. G. H. Johnson, and Manning A. Smith*. May 1961. 35 pp.
- No. 2. Fishery statistical districts of the Great Lakes, by *Stanford H. Smith, Howard J. Buettner, and Ralph Hile*. September 1961. 24 pp.
- No. 3. Commercial fish production in the Great Lakes 1867-1977, by *Norman S. Baldwin, Robert W. Saalfeld, Margaret A. Ross, and Howard J. Buettner*. September 1979. 187 pp. (Supersedes 1962 edition and 1970 supplement.)
- No. 4. Estimation of the brook and sea lamprey ammocete populations of three streams, by *Bernard R. Smith and Alberton L. McLain*. September 1962. pages 1-18.  
A photoelectric amplifier as a dye detector, by *Wesley J. Ebel*. September 1962. pages 19-26.
- No. 5. Collection and analysis of commercial fishery statistics in the Great Lakes, by *Ralph Hile*. December 1962. 31 pp.
- No. 6. Limnological survey of Lake Erie 1959 and 1960, by *Alfred M. Beeton*. November 1963. 32 pp.
- No. 7. The use of alkalinity and conductivity measurements to estimate concentrations of 3-trifluoromethyl-4-nitrophenol required for treating lamprey streams, by *Richard K. Kanayama*. November 1963. 10 pp.
- No. 8. Synergism of 5,2'-dichloro-4'-nitro-salicylanilide and 3-trifluoromethyl-4-nitrophenol in a selective lamprey larvicide, by *John H. Howell, Everett L. King, Jr., Allen J. Smith, and Lee H. Hanson*. May 1964. 21 pp.
- No. 9. Detection and measurement of organic lampricide residues, by *Stacy L. Daniels, Lloyd L. Kempe, Thomas J. Billy, and Alfred M. Beeton*. 1965. 18. pp.
- No. 10. Experimental control of sea lampreys with electricity on the south shore of Lake Superior, 1953-60, by *Alberton L. McLain, Bernard R. Smith, and Harry H. Moore*. 1965. 48 pp.
- No. 11. The relation between molecular structure and biological activity among mononitrophenols containing halogens, by *Vernon C. Applegate, B. G. H. Johnson, and Manning A. Smith*. December 1966. pages 1-19.  
Substituted nitrosalicylanilides: A new class of selectively toxic sea lamprey larvicides, by *Roland J. Starkey and John H. Howell*, December 1966. pages 21-29.
- No. 12. Physical limnology of Saginaw Bay, Lake Huron, by *Alfred M. Beeton, Stanford H. Smith, and Frank F. Hooper*. September 1967. 56 pp.
- No. 13. Population characteristics and physical condition of alewives, *Alosa pseudoharengus*, in a massive dieoff in Lake Michigan, 1967, by *Edward H. Brown, Jr.* December 1968. 13 pp.
- No. 14. Limnological survey of Lake Ontario, 1964 (five papers), by *Herbert E. Allen, Jerry F. Reinwand, Roann E. Ogawa, Jarl K. Hiltunen, and LaRue Wells*. April 1969. 59 pp.
- No. 15. The ecology and management of the walleye in western Lake Erie, by *Henry A. Regier, Vernon C. Applegate and Richard A. Ryder*, in collaboration with *Jerry V. Manz, Robert G. Ferguson, Harry D. Van Meter, and David R. Wolfert*. May 1969. 101 pp.
- No. 16. Biology of larval sea lampreys (*Petromyzon marinus*) of the 1960 year class, isolated in the Big Garlic River, Michigan, 1960, by *Patrick J. Manion and Alberton L. McLain*. October 1971. 35 pp.
- No. 17. New parasite records for Lake Erie fish, by *Alex O. Dechtiar*. April 1972. 20 pp.
- No. 18. Microbial degradation of the lamprey larvicide 3-trifluoromethyl-4-nitrophenol in sediment-water systems, by *Lloyd L. Kempe*. January 1973. 16 pp.
- No. 19. Lake Superior—A case history of the lake and its fisheries, by *A. H. Lawrie and Jerold F. Rahrer*. January 1973. 69 pp.
- No. 20. Lake Michigan—Man's effects on native fish stocks and other biota, by *LaRue Wells and Alberton L. McLain*. January 1973. 55 pp.
- No. 21. Lake Huron—The ecology of the fish community and man's effects on it, by *A. H. Berst and G. R. Spangler*. January 1973. 41 pp.
- No. 22. Effects of exploitation, environmental changes, and new species on the fish habitats and resources of Lake Erie, by *W. L. Hartman*. April 1973.

NATIONAL SEA GRANT DEPOSITORY

DATE: OCT. 20 1987

- No. 23. A review of the changes in the fish species composition of Lake Ontario, by *W. J. Christie*. January 1973. 65 pp.
- No. 24. Lake Opeongo—The ecology of the fish community and of man's effects on it, by *N. V. Martin* and *F. E. J. Fry*. March 1973. 34 pp.
- No. 25. Some impacts of man on Kootenay Lake and its salmonoids, by *T. G. Northcote*. April 1973. 45 pp.
- No. 26. Control of the sea lamprey (*Petromyzon marinus*) in Lake Superior, 1953–70, by *Bernard R. Smith*, *J. James Tibbles*, and *B. G. H. Johnson*. March 1974. 60 pp.
- No. 27. Movement and recapture of parasitic-phase sea lampreys (*Petromyzon marinus*) tagged in the St. Marys River and Lakes Huron and Michigan, 1963–67, by *Harry H. Moore*, *Frederick H. Dahl*, and *Aarne K. Lamsa*. July 1974. 19 pp.
- No. 28. Changes in the lake trout population of southern Lake Superior in relation to the fishery, the sea lamprey, and stocking, 1950–70, by *Richard L. Pycha* and *George R. King*. July 1975. 34 pp.
- No. 29. Chemosterilization of the sea lamprey (*Petromyzon marinus*), by *Lee H. Hanson* and *Patrick J. Manion*. July 1978. 15 pp.
- No. 30. Biology of larval and metamorphosing sea lampreys (*Petromyzon marinus*) of the 1960 year class in the Big Garlic River, Michigan, Part II, 1966–72, by *Patrick J. Manion* and *Bernard R. Smith*. October 1978. 35 pp.
- No. 31. Walleye stocks in the Great Lakes, 1800–1975; fluctuations and possible causes, by *J. C. Schneider* and *J. H. Leach*. February 1979. 51 pp.
- No. 32. Modeling the western Lake Erie walleye population: a feasibility study, by *B. J. Shuter*, *J. F. Koonce*, and *H. A. Regier*. April 1979. 40 pp.
- No. 33. Distribution and ecology of lampreys in the Lower Peninsula of Michigan, 1957–75, by *Robert H. Morman*. April 1979. 59 pp.
- No. 34. Effects of granular 2', 5-dichloro-4'-nitrosalicylanilide (Bayer 73) on benthic macroinvertebrates in a lake environment, by *Philip A. Gilderhus*. May 1979. pages 1–5.  
Efficacy of antimycin for control of larval sea lampreys (*Petromyzon marinus*) in lentic habitats, by *Philip A. Gilderhus*. May 1979. pages 6–17.
- No. 35. Variations in growth, age at transformation, and sex ratio of sea lampreys reestablished in chemically treated tributaries of the upper Great Lakes, by *Harold A. Purvis*. May 1979. 36 pp.
- No. 36. Annotated list of the fishes of the Lake Ontario watershed, by *E. J. Crossman* and *Harry D. Van Meter*. December 1979. 25 pp.
- No. 37. Rehabilitating Great Lakes ecosystems, edited by *George R. Francis*, *John J. Magnuson*, *Henry A. Regier* and *Daniel R. Talhelm*. December 1979. 99 pp.
- No. 38. Green Bay in the future—a rehabilitative prospectus, edited by *Hallett J. Harris*, *Daniel R. Talhelm*, *John J. Magnuson*, and *Anne M. Forbes*. September 1982. 59 pp.
- No. 39. Minimum size limits for yellow perch (*Perca flavescens*) in western Lake Erie, by *Wilbur L. Hartman*, *Stephen J. Nepszy*, and *Russell L. Scholl*. March 1980. 32 pp.
- No. 40. Strategies for rehabilitation of lake trout in the Great Lakes: proceedings of a conference on lake trout research, August 1983, edited by *Randy L. Eshenroder*, *Thomas P. Poe*, and *Charles H. Olver*. August 1984. 63 pp.
- No. 41. Overfishing or pollution? case history of a controversy on the Great Lakes, by *Frank N. Egerton*. January 1985. 28 pp.
- No. 42. Movement and capture of sea lampreys (*Petromyzon marinus*) marked in northern Lake Huron, 1981–82, by *John W. Heinrich*, *William C. Anderson*, and *Susan D. Oja*. February 1985. pages 1–14.  
Response of spawning-phase sea lampreys (*Petromyzon marinus*) to a lighted trap, by *Harold A. Purvis*, *Clarence L. Chudy*, *Everett L. King, Jr.* and *Verdel K. Dawson*. February 1985. pages 15–25.
- No. 43. A prospectus for the management of the Long Point ecosystem, by *George R. Francis*, *A. P. Lino Grima*, *Henry A. Regier*, and *Thomas H. Whillans*. March 1985. 109 pp.
- No. 44. Population dynamics and interagency management of the bloater (*Coregonus hoyi*) in Lake Michigan, 1967–1982, by *Edward H. Brown, Jr.*, *Ronald W. Rybicki*, and *Ronald J. Poff*. April 1985. 34 pp.
- No. 45. Review of fish species introduced into the Great Lakes, 1819–1974, by *Lee Emery*. April 1985. 31 pp.