This is to certify that the

thesis entitled
HISTOPATHOLOGY OF
SEA LAMPREY (PETROMYZON MARINUS)
WOUNDS IN RAINBOW TROUT

presented by

Ronald E. Kinnunen

has been accepted towards fulfillment
of the requirements for

M. S. degree in Fisheries and Wildlife

Harvard E. Johnson
Major professor

Date July 27, 1979

O-7639

HISTOPATHOLOGY OF SEA LAMPREY (PETROMYZON MARINUS) WOUNDS IN RAINBOW TROUT

By

Ronald E. Kinnunen

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1979

ABSTRACT

HISTOPATHOLOGY OF SEA LAMPREY (PETROMYZON MARINUS) WOUNDS IN RAINBOW TROUT

By

Ronald E. Kinnunen

Relatively little is known of the pathology of sea lamprey wounds in fish. Although lamprey attacks are frequently lethal, some fish survive multiple wounds as evidenced by scars found on larger trout and salmon.

This study describes the histopathology of sea lamprey wounds in rainbow trout. Fish held in the laboratory were subjected to lamprey attack for prescribed time periods to provide wound samples of different ages up to ten days. A lamprey destroys the epidermis in as little as four hours, penetrates the dermis from twelve hours to two days, and inflicts a large area of muscle damage from two to ten days.

The wound healing process was examined by collecting tissue samples of the healing wound at prescribed times up to three months. Reepithelialization of the wound area was complete by two weeks at which time heavy fibrogranulation tissue encompassed the area of dermal penetration and damaged muscle. The wound area had not returned to normal by three months as was evidenced by the fibroblastic activity which remained.

A secondary objective of the study was to determine the impact of sea lamprey attack on the hemopoietic tissues of the kidney and spleen and on the circulating blood. The

blood parameters which were monitored during wound development and wound healing included the hematocrit, hemoglobin, RBC precursers, leucocrit, and leucocyte differential. The spleen was the only hemopoietic organ which exhibited pathological changes.

DEDICATION

To my parents, Irene and William Kinnunen

ACKNOWLEDGMENTS

I wish to thank the following for their advice and assistance during my Master's program:

Special thanks to Dr. Joseph B. Hunn and Everett Louis King of the Hammond Bay Biological Station (U.S.F.W.S.). Sea lamprey and the photograph of the oral disk of a lamprey (Figure 9) were provided by the Hammond Bay Biological Station.

Dr. Eugene W. Roelofs and Dr. Niles R. Kevern for serving on my graduate committee.

Dr. Charles K. Whitehair for his encouragement in the area of fish pathology.

Dr. Howard D. Stowe for the use of his laboratory for histological work, reviewing my thesis, and serving as a member of my graduate committee.

Barbara Goelling for her assistance and advice during histological preparation of the tissues.

Dr. Vance L. Sanger for his advice in the hematological work and reviewing my thesis.

Dr. Howard E. Johnson for his never ending patience and constructive criticism as he served as committee chairman and guided my research.

Terry Aiken for the excellent graphic work incorporated within the thesis.

Theron K. Anderson for his expert advice in photography.

My sister, Helen Peters, for a job well done in reviewing the grammar within the thesis.

This study was funded in part by the Michigan Agricultural Experiment Station.

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INTRODUCTION

The decline in major fish populations in the Great Lakes has been attributed in part to parasitism by the sea lamprey (Petromyzon marinus). Although chemical control has successfully reduced sea lamprey populations, the incidence of scarred fish taken in field surveys and by sport fishermen indicate significant numbers of lamprey remain in the Great Lakes.

Relatively little is known of the pathology of lamprey wounds in fish. Although lamprey attacks are frequently lethal, some fish survive multiple wounds as evidenced by scars found on larger trout and salmon.

There is a need for information on the histopathology of sea lamprey wounds in fish and the wound healing process. In addition to improving our knowledge of the host-parasite relationship, the data gathered should aid in the determination of the age of wounds and scars found in field surveys.

This study was proposed to describe the histopathology of sea lamprey wounds in rainbow trout. Fish held in the laboratory were subjected to lamprey attacks for prescribed time periods to provide wounds at various stages. This allows observations to be made on the areas of the integument and underlying muscle which were affected as the wound progressed, and to note the extent of damage that occurred.

Histopathological sections were also prepared from trout that had healed for prescribed time periods to provide information on the wound healing process. This type of information will determine if a trout that has been attacked by a lamprey and later escapes actually heals and thus may survive.

A second objective was to determine the impact of sea lamprey attack on the hemopoietic tissues of the kidney and spleen and on the circulating blood. Since the majority of the sea lamprey's diet consists of blood and the fish has a relatively small blood volume, this blood loss could result in drastic changes in various blood parameters. By monitoring various blood parameters over 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. Since the hemopoietic centers of the kidney and spleen will be taxed during lamprey feeding, the pathology of these were studied to determine what effects, if any, may occur.

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METHODS AND MATERIALS

Experimental Animals

The rainbow trout (Salmo gairdneri) used in the study averaged 31 cm total length and were obtained from the Midwest Trout Farm, Harrison, Michigan. Prior to experimentation the fish were held in 1115 liter circular tanks which received flowing 12°C well water of pH 7.1, hardness 330 mg $CaCO_3$/1 and alkalinity 325 mg $CaCO_3$/1. Dissolved oxygen was 9.0 ppm or higher. The fish were fed a commercial trout diet ad libitum.

The sea lamprey obtained from the United States Fish and Wildlife Service Laboratory at Hammond Bay, Michigan were recently transformed feeders which averaged 15 cm in total length. The lamprey were maintained in 146 liter tanks receiving flowing well water and allowed to feed on carp (Cyprinus carpio) prior to experimentation.

Experimental Procedure for Wound Development

The sequential development of lamprey wounds was followed by collecting wounded fish at 4 hours, 12 hours, 2 days and 10 days after the initial lamprey attachment. Five fish were used for each sampling period. An individual trout was weighed and measured and placed in a 146 liter fiberglass tank with several sea lamprey. After one of the lamprey had attached itself to the trout, the time and

position of attachment were noted and the remaining lamprey were removed to a holding tank. If a sea lamprey attached to the head region, it was detached immediately because study of the pathology of this region was not intended. The lamprey was allowed to feed on the trout for a prescribed time period after which the lamprey and trout were separated by anesthesia in a MS-222 solution (80 ppm), and then both were weighed and measured. The wound was then measured and photographed as a record of gross pathology.

Laboratory Procedure

A blood sample was collected from the caudal artery of the trout with a 3.8 cm 22 gauge needle attached to a 3.0 cc syringe and no more than 0.5 cc of blood was collected at one time. The blood was placed into 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 while the other was fixed in absolute methanol for a spare. The stained smear was later examined under oil immersion (1000x) for immature red and white blood cell differential counts. The immature red blood cells were determined as a percentage of the first 500 red blood cells counted. The white blood cell differential included the differentiation of the first 100 cells counted as to lymphocytes, thrombocytes, and granulocytic, metagranulocytic, immature, or segmented neutrophils (Lehmann and Sturenberg, 1975).

The 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.

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

Histopathologic Technique

A tissue sample was taken by cutting a rectangular section across the wound area and samples of the spleen and anterior kidney were removed and fixed immediately in buffered neutral formalin solution. The tissue samples were embedded in paraffin, sectioned and stained with H & E. For some wound sections that exhibited fibrogranulation, a second slide was prepared and stained with Mallory's trichrome to demonstrate fibrosis.

Experimental Procedure for Wound Healing

The wound healing process was studied by collecting samples of wounded fish at 2 days, 2 weeks, 1 month and 3 months after lamprey detachment. Five fish were used in each sample group. Lamprey were allowed to feed on trout for an 8 day time period, separated by anesthesia in MS-222 and then returned to holding tanks for the prescribed time period. 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 and the wound photographed at the time of each blood collection.

Statistical Procedure for Blood Data

The blood values were subjected to t-tests for the difference between two means and for paired comparisons (Sokal and Rohlf, 1973) and differences were considered significant if $P<.05$.

Feeding Activity of Fish

All fish were fed ad libitum during the sea lamprey attachment except those of the 4 hour, 12 hour, and 2 day attachment periods which were not fed. Records were kept on the feeding activity of these fish and also on the group held during wound healing.

RESULTS AND DISCUSSION

Size of Trout, Lamprey and Inflicted Wound

The average size of the trout and sea lamprey, and the size of the inflicted wound in both the wound development and wound healing studies are given in Table 1. The wound development phase of the study was completed prior to the initiation of the wound healing study. All lamprey were actively growing and the size of the lamprey used in the wound healing study was therefore larger (22.9 cm) than those of the wound development study (19.1 cm). The oral disk wound diameters had an overall average of 2.10 x 1.83 cm in the wound development group compared to 2.62 x 2.15 cm in the wound healing group.

The sea lamprey used were recently transformed feeders, were actively growing and although small, they inflicted severe and, at times, lethal wounds. Lennon (1954) found that sea lamprey wound dimensions were not reliable for determining the lethality on host fishes because the small wounds made by the newly transformed feeders had the same mortal results for their host as the more extensive wounds made by large lamprey.

Weight Loss of Fish During Lamprey Attachment

The weight loss data for fish during lamprey attachment are given in Table 1. The percent weight loss after ten days

Table 1. Measurements of fish, lamprey, and inflicted wound in the wound development and wound healing groups $\bar{X}$ ±S.E.

Time	Preattachment Fish Length (cm)	Preattachment Fish Weight (g)	Lamprey at Detachment Length (cm)	Lamprey at Detachment Weight (g)	% wt. loss of fish During Lamprey Attachment	Oral Disk Wound Diameter (cm)
WOUND DEVELOPMENT						
0 hours	30.4±0.5	308.0±15.9	---	---	---	---
4 hours	30.2±0.5	not obtained	19.1±0.0	12.8±0.2	---	2.18 x 1.76
12 hours	31.2±0.4	299.9±16.1	18.6±0.5	11.1±1.1	3.0±0.2	1.80 x 1.76
2 days	30.7±0.2	313.3±4.2	18.9±0.4	13.3±0.8	4.0±0.4	2.14 x 1.98
10 days	30.2±0.5	314.2±14.0	20.1±0.5	16.6±1.8	3.8±1.0	2.28 x 1.84
Grand Avg.	30.6	309.1	19.1	13.5		2.10 x 1.83
WOUND HEALING						
2 days	31.1±0.9	331.0±27.5	25.6±1.1	34.1±5.0	3.3±0.5	3.28 x 2.44
2 weeks	31.2±0.5	337.9±13.3	22.8±1.0	25.0±3.7		2.30 x 1.96
1 month	30.8±0.3	317.8±15.7	21.6±1.1	21.8±3.5		2.34 x 2.06
3 months	31.5±0.5	327.1±12.3	21.6±1.0	20.1±2.8		2.56 x 2.16
Grand Avg.	31.1	328.4	22.9	25.3		2.62 x 2.15

of lamprey attachment was somewhat lower than that of the two day lamprey attachment. This could be due to the fact that the fish in the ten day attachment group were accepting feed during the attachment period while those in the two day lamprey attachment group did not receive feed. The weight loss of four control fish initially weighing 281.9±16.4 g which were not fed was 2.6±0.1% and 3.6±0.1% at 24 and 48 hours respectively. These values were lower than fish which were subjected to comparable lamprey attachment times.

The fish of the wound healing group all experienced an 8 day sea lamprey attachment and a 3.3±0.5% weight loss occurred over this period. Twenty-one percent of the fish refused feed by day 1 of the lamprey attachment and by day 7 of lamprey attachment 52.6% of the fish refused feed.

Weight loss occurring in fish during lamprey attachment could be attributed in part to fish either refusing or reducing food intake. But the loss of blood and body fluids from the host fish during lamprey attack probably had an important influence on the amount of weight loss. Farmer et al. (1975) determined that the initiation of feeding by the lamprey on the host was found to commence within 6 hours of attachment.

Percent Weight and Length Increases in Wound Healing Fish

The percent length and weight increases of fish undergoing wound healing are shown in Figure 1. At 2 days after lamprey detachment the rate of weight increase was reduced,

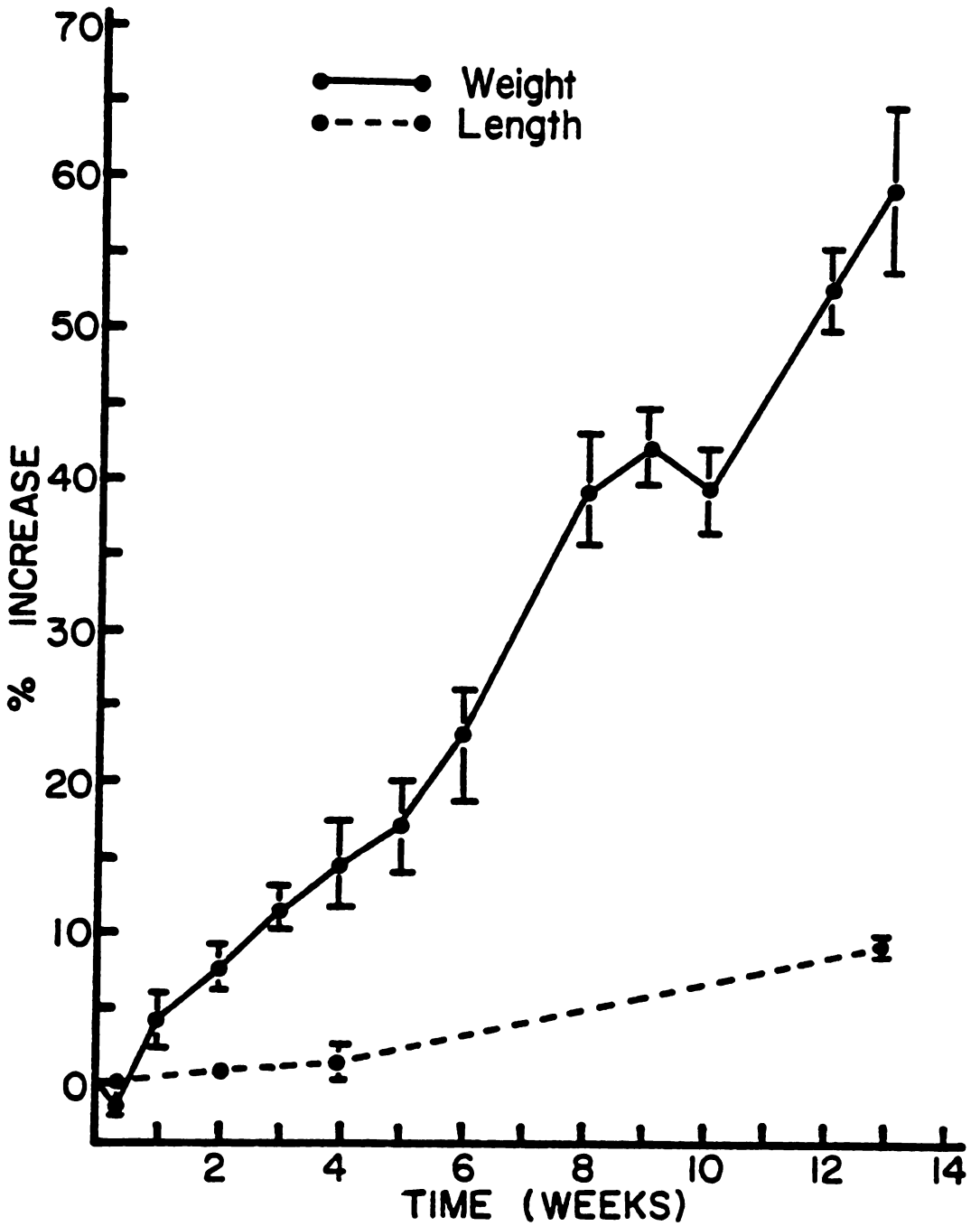

Figure 1. Percent weight and length increases in wound healing fish

probably due to the fact that most of the fish in this group were refusing feed. The weight then progressively increased to week 9 before dropping slightly at week 10. This drop could be attributed to the fighting that occurred among these fish as they reached sexual maturity. After week 10, the weight increased progressively to week 13 at which time the last group of fish was sampled. The fish length increased throughout the wound healing time period.

Areas of Wounding

The sea lamprey attachment locations were plotted by percent occurrence over different body regions of the fish (Figure 2). Twenty-five percent of attachments occurred in each of regions II and III, 19.8% in region VI, 15.6% in region IV, 10.4% in region V, and 4.2% in region I. Half of all the lamprey attachments occurred in regions II and III.

Lethal sea lamprey attachments by percent occurrence were plotted over different body regions of the fish (Figure 3), which represents 19.5% of the fish which were attacked. The distribution of lethal wounds was: 8.7% in region III, 4.3% in each of regions II and IV, and 2.2% in region VI. No lethal wounds occurred in area V. Lamprey that attached to region I were removed so no mortal wounds occurred in this area. The highest mortality occurred from attacks in region III followed by regions II and IV. Because these were timed experiments, many of the lamprey attachments were terminated before death ensued and thus

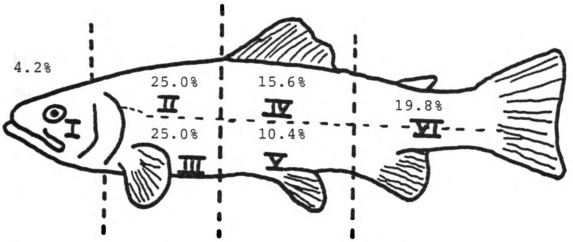

Figure 2. Sea lamprey attachment location by percent occurrence

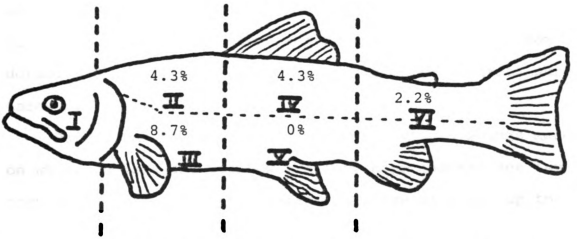

Figure 3. Sea lamprey lethal attachment location by percent occurrence

higher lethal percentages in other body regions would probably have occurred if given sufficient time.

Lennon (1954) working with sea lamprey scarring on rainbow trout in the laboratory found the incidence of scarring to be 2.7% in region I, 29.8% in region II, 23.9% in region III, 21.2% in region IV, 12.9% in region V, and 9.3% in region VI. The most striking difference between his results and the present study is the lower number of wounds found in region VI. Lennon's results show a 10.5% lower occurrence of wounds in this region.

Lennon (1954) examined 20 species of fish that were attacked by sea lamprey in the laboratory or in Lake Huron and found the upper half of the middle third of the fish's body was most often attacked on aquarium hosts, and the wounds occurred most often on the lower half of the middle third of the fish in the lake. Since all wounds were available for study in the laboratory, and scarred fish from the lake only included fish that had survived lamprey attacks, it was determined that there was a greater mortality from dorsal and head attacks than by attacks on the ventral surfaces of the fish.

Parker and Lennon (1956) found that single wounds made on white suckers and rainbow trout by experimental sea lamprey at any stage of their parasitic phase of life, up to full maturity, may be lethal on any part of the body except fins. They also found that the distribution of attacks conformed to those described by Lennon (1954).

Percent Weight Change of Mortally Wounded Fish

The sizes of mortally wounded trout, attacking lamprey, and inflicted lethal wounds are given in Table 2. The sea lamprey attachment time before death varied from less than 36 hours to 8 days and most fish either refused feed during lamprey attachment or were off feed by day 3 of lamprey attachment.

Sea lamprey inflicted lethal wounds on 19.5% of their hosts during experimentation. Some of the fish died a few hours before they were discovered and removed. These fish all experienced weight gains which may or may not be due to a loss of osmoregulation (Table 2). Lennon (1954) observed that injury to the integument upsets the osmotic balance between exposed tissue and lake water and frequently results in a "waterlogged" condition in white suckers and at times in trout in laboratory aquaria. Fish weight gains in fresh water have been noted to occur in stress situations (Westfall 1943; Meyer et al. 1956; Rao 1969; Houston et al. 1971; Stevens 1972; Kirk 1974). Only one other fish, sampled because it was near death, demonstrated a weight gain; the others experienced weight losses (Table 2).

Gross Pathology

Wound Development

The area of the lamprey wound was generally elevated with a distinct central zone where the tongue had penetrated the skin.

Table 2. Measurements of mortally wounded fish, lamprey, and inflicted wound.

Attachment Time Before Death	Preattachment Fish		Percent Weight change of fish at Detachment	Lamprey at detachment		Oral disk wound Diameter (cm)
	Length (cm)	Weight (g)		Length (cm)	Weight (g)	
<36 hours †	33.0	374.5	+2.9	28.5	41.0	2.6 x 2.2
<36 hours †	32.0	368.4	+3.1	25.0	36.0	2.4 x 2.4
<4.5 days †	31.5	358.2	+4.7	26.0	32.4	2.4 x 2.4
5 days *	32.0	402.9	+2.7	27.0	37.7	multiple wounds
<6 days †	29.0	292.3	+2.1	24.0	28.5	2.4 x 2.4
7 days *	31.5	370.0	-1.7	27.0	38.5	3.0 x 2.6
8 days †	30.0	328.8	- -	27.5	42.0	3.0 x 3.0 3.0 x 2.5
8 days **	32.0	362.2	-4.2	25.5	34.0	2.5 x 2.4
8 days ***	31.5	322.0	-3.3	24.5	27.1	3.5 x 3.5

† Fish were found dead and may have been in the water a few hours following death.

* Fish were near death and were sampled at this time.

** Fish experienced an 8 day lamprey attachment and died within 16 hours of detachment.

*** Fish experienced an 8 day lamprey attachment and was sampled 10 days following detachment because fish was near death.

Four Hour Wound: Wounds were elevated with one to three white abraded areas averaging 2.0 mm in diameter where the lamprey's tongue had penetrated the skin into the upper depths of the dermis. The peripheral part of the wound, which had been covered by the oral disk of the lamprey, lacked scales and showed slight to dark discoloration - at times a brown color in the area was evident (Figure 4).

Twelve Hour Wound: The central portion had not significantly enlarged (average 2.0 mm) but the depth of the wounds had increased with penetration to the lower dermis and in one case into the muscle. Petechial hemorrhaging was evident on one fish and a more severe hemorrhaging occurred where the tongue had penetrated to the muscle. The peripheral portion of the wound was lacking scales and showed discoloration - also at times a dark brown color was evident (Figure 5).

Two Day Wound: The central portion of the wounds had increased slightly ranging from 2.0 to 3.0 mm where the lamprey's tongue had penetrated through the dermis to the muscle. The peripheral portion of the wound area was lacking scales and exhibited dark discoloration with some white interlaced areas of abrasion, with the same brown coloration of the area being evident at times (Figure 6).

Ten Day Wound: The central portion of the wounds had one to three areas of dermal penetration to the muscle which ranged from 2.0 to 6.0 mm across with some ulceration occurring in the necrotic muscle in the larger dermal openings.

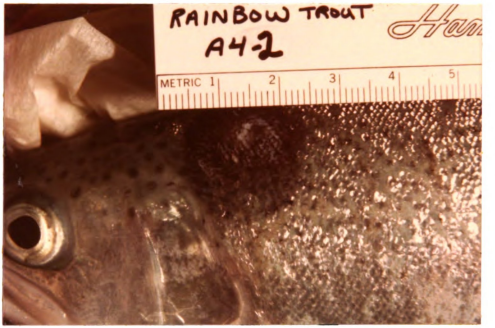

Figure 4. Sea lamprey wound on a rainbow trout four hours after attachment

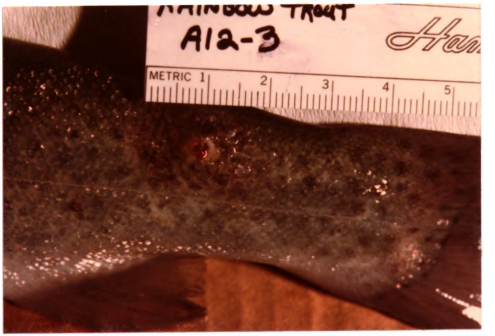

Figure 5. Sea lamprey wound on a rainbow trout twelve hours after attachment

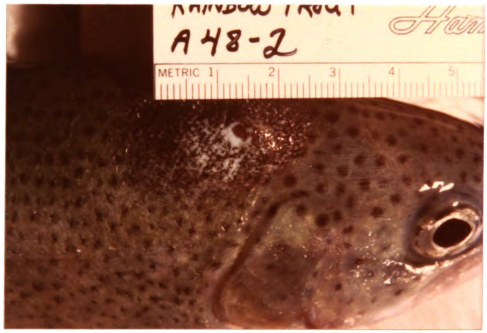

Figure 6. Sea lamprey wound on a rainbow trout two days after attachment

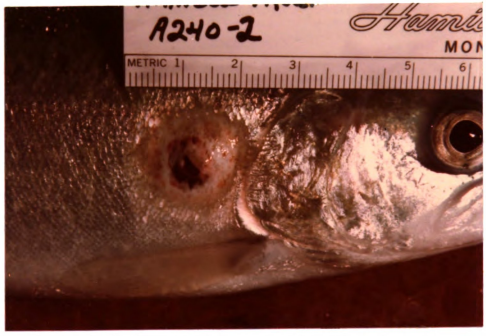

Figure 7. Sea lamprey wound on a rainbow trout ten days after attachment

The peripheral portion of the wound was lacking scales and was bleached out to a white abraded area which frequently showed petechial hemorrhaging. At times a few small penetrations through the dermis to the muscle were evident (Figure 7).

During wound development all of the wounds were elevated which may be attributed to the suction exerted by the sea lamprey on the skin of the host fish (Figure 8). The first sign that the lamprey's tongue had penetrated the dermis was at 12 hours. This is probably the time when the most active feeding would occur as a richer blood supply would be available below the dermis. The dermal penetration by the tongue became larger from 2 to 10 days, and by 10 days some of the penetrations were 6.0 mm across. Loss of scales usually occurred as early as 4 hours. The wounds progressively became more severe as the time of attachment increased. The abraded area of the wound expanded until 10 days when it was bleached out and showed petechial hemorrhaging. Aside from the tongue penetration through the dermis at 10 days which exposed ulcerated muscle, other small penetrations through the dermis were evident which could be attributed to the teeth within the oral disk of the lamprey pressing hard against the fish's body (Figure 9).

Lennon (1954) found that a sea lamprey attaches to a fish by strong suction which brings the skin of its host into contact with the lamprey's armed tongue. A rocking motion of the armed tongue then rasps a feeding hole through the skin and tissues of the host. This motion continues

Figure 8. Sea lamprey attached to a rainbow trout

Figure 9. Oral disk of a sea lamprey

until a flow of blood is obtained. He also observed that many new wounds show the application and pattern of buccal dentation. The suction exerted brings the disk teeth into close application with the surface of the fish, and there the cusps penetrate the skin or scales to help anchor the lamprey.

Lennon (1954) measured the diameter and depth of the wound hole on lake and laboratory fishes and found no definite relation to the size of the lampreys that made the wounds. He found the area and depth of the wound hole depended on the attack duration, the amount of rasping by the tongue, the extent of tissue lysis caused by the action of the buccal gland secretion, the attack location on fish and the amount of movement by the lamprey from the original site of attachment.

Wound Healing

Wounds at Lamprey Detachment: The wounds were elevated with the central portion exhibiting an average lesion of 6.0 mm by 4.0 mm across where the lamprey's tongue penetrated the dermis to the muscle (Figure 10). These dermal penetrations ranged from 1.0 mm by 1.0 mm to 15.0 mm by 6.0 mm across and only two fish lacked complete dermal penetration at the time of lamprey detachment. Some ulceration into the necrotic muscle occurred in the larger dermal openings and the muscle varied from pale to dark in appearance and was, at times, hemorrhagic. The peripheral part of the wound area was a white abraded area which at times had some grey

or brown pigmentation remaining. At times multiple perforations through the dermis to the muscle were evident. Hemorrhaging, mainly petechial, along with some erythema was evident on many fish in the oral disk region of the wound.

Two Days: Many of the wounds decreased in elevation and some sloughing of tissue in the white abrased central region was evident, especially around the dermal penetration. Most of the necrotic muscle was pale in color and at times was hemorrhagic. Some petechial hemorrhages and erythema were present in the peripheral portion of the wound area.

One Week: A decline in the elevation of some of the wounds was evident and the area of dermal penetration expanded by 1.0 to 2.0 mm (Figure 11). Most of the necrotic muscle was dark colored and somewhat hemorrhagic. The peripheral portion of the wound area frequently became more white or bleached out and at times had some grey or brown pigmentation remaining. Small perforations through the dermis which were present at times in this area were slightly enlarged. Hemorrhaging, mainly petechial, along with some erythema, was also apparent in the peripheral portion of the wounds on many fish.

Two Weeks: Wound elevation was no longer evident on any of the fish and the area of dermal penetration had enlarged slightly. Most of the necrotic muscle visible through the dermal opening was dark in color and this ulcerated area in the muscle was shallower than in the previous week. The peripheral portion of the wound area was white in appearance

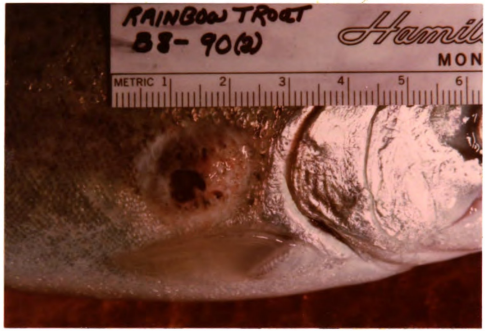

Figure 10. Eight day sea lamprey wound on a rainbow trout

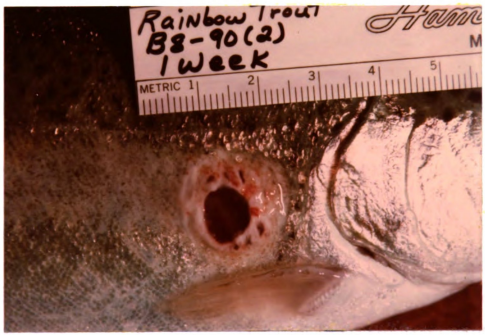

Figure 11. Eight day sea lamprey wound after one week of wound healing

with some petechial hemorrhages evident. Some of the fish that had brown pigmentation in parts of the wound area had a grey color replacement. One fish that lacked a complete dermal penetration had a dark grey to black pigmentation returning in most of the wound area.

Three Weeks: The area of dermal penetration closed an average of 1.0 mm from the previous week. A glassy covering was evident over the wound area in many of the fish and dark necrotic muscle was present below this in the area of the dermal opening. The peripheral portion of the wound had grey, and at times brown pigmentation, which had begun to move into the whitened area from the wound perimeter. The small dermal perforations that were present in this area in some of the fish had started to close and most of the petechial hemorrhage which was present in the previous week was no longer evident. When the wound occurred on the ventral surface of the fish, in the less pigmented region, the wound area appeared whiter than the surrounding area. The fish that lacked a complete dermal penetration had dark grey to black pigmentation present in most of the wound area as well as scale reestablishment.

Four Weeks: The area of dermal penetration closed another 0.5 to 3.0 mm from the previous week (Figure 12). A glassy covering was present over the wound area in many of the fish which took on a greyish sheen over the once exposed muscle. At times, the muscle was no longer visible through the dermal opening. The peripheral portion of the wound area

had a dark grey, and at times brown pigmentation, which occupied a larger part of the wound area in comparison to the previous week. Wounds that occurred in the ventral region of the body were still lighter colored than the surrounding area. One of the fish that lacked a complete dermal penetration had dark grey to black pigmentation present in the wound area.

Weeks Five through Six: The dermal penetration had closed another 0.5 to 2.0 mm since week 4 (Figure 13). A glassy covering took on a greyish sheen over the once exposed muscle making it no longer visible. The peripheral portion of the wound area was almost entirely occupied by dark grey or greyish-brown pigmentation with a few white areas remaining. Some petechial hemorrhage had shown up in one of the wounds at week 5 but was gone again by week 6. Some fish had reestablishment of scales in the wound area, especially around the wound perimeter. A wound that occurred in the non-pigmented region of the body was still a lighter color than the surrounding area.

Weeks Eight through Ten: A greyish indentation averaging 1.5 by 3.0 mm across was all that remained where the lamprey tongue had penetrated through the dermis. The peripheral portion of the wound area had become more densely pigmented with grey until nearly all the wound area was covered. Scales were still coming into most of the wounds but were still lacking in much of the wound area. One fish, which had a wound inflicted in the non-pigmented region of

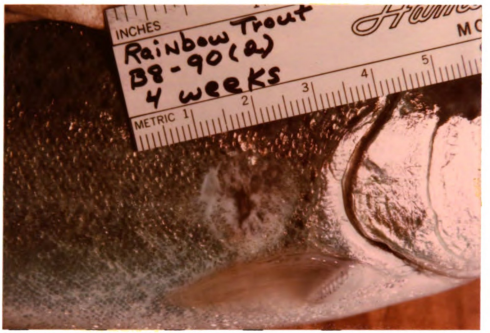

Figure 12. Eight day sea lamprey wound after four weeks of wound healing

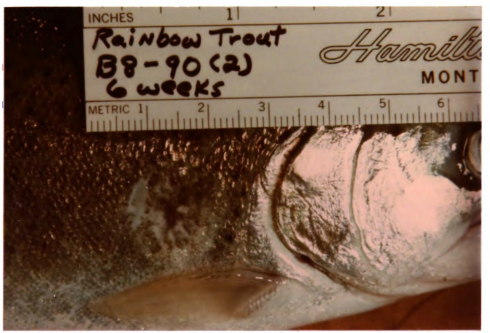

Figure 13. Eight day sea lamprey wound after six weeks of wound healing

the body, had exhibited complete closure of the central part of the wound and this area had progressively become whiter in this region over these weeks. The peripheral portion of this wound was whiter than the surrounding area. Another fish which lacked a complete dermal penetration had a slightly darker grey pigmentation in the wound area compared to the surrounding area.

Weeks Twelve through Thirteen: A grey shallow indentation averaging 1.3 by 2.5 mm across remained where the lamprey tongue had penetrated the dermis (Figure 14). The peripheral portion of the wound had regained most of its grey pigmentation and, in most of the wounds, scales were still reestablishing but were lacking in much of the wound area. One fish, which had been wounded in the non-pigmented region of the body, had become whiter in the area of the dermal closure in the central area of the wound, and the peripheral portion of the wound was whiter than the surrounding area. The fish that lacked a complete dermal penetration had a slightly darker grey pigmentation in the wound area in comparison to the surrounding area.

A sea lamprey, while attached to the host fish, is not always stationary and is known to move over the host's body while attached. In one case, a sea lamprey moved about 8.0 cm from the mid-region to behind the operculum within 24 hours, leaving a trail of dark grey to brown pigmentation in the movement line which was void of scales. In two weeks, this area appeared to be more bleached out and white in

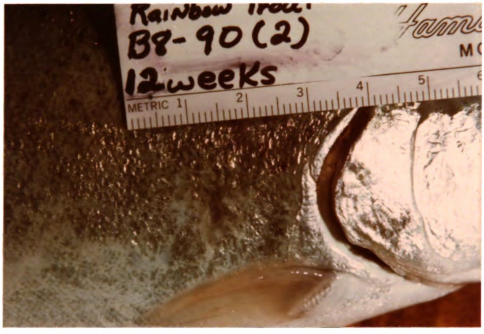

Figure 14. Eight day sea lamprey wound after twelve weeks of wound healing

Figure 15. Area of sea lamprey movement over the host's body two weeks after initial movement

color (Figure 15). By week five, this area had grey pigmentation and scale reestablishment, and by week seven, this area was not noticeably different from the surrounding area.

Many of the wounds that were inflicted on fish in the wound healing group at lamprey detachment were elevated with the centers exhibiting an average lesion of 6.0 by 4.0 mm across where dermal penetration to the muscle occurred. Only two fish did not have complete dermal penetration at the time of lamprey detachment. Similar incomplete wounds, as noted by Lennon (1954) varied from temporary marks on the surface of the fish to wounds in which the scales had been torn but the skin had not been penetrated. Farmer et al. (1975) reported that in some cases sea lampreys attached to trout and then detached without feeding.

The peripheral area of the wound was generally bleached out in the abraded area and at times had small perforations through the dermis. These perforations were likely caused by the teeth within the oral disk of the lamprey. Petechial hemorrhaging and some erythema also occurred in the peripheral area of the wound.

The wound elevation was diminished 2 weeks after lamprey detachment and the areas of the dermal penetration had enlarged. This could be attributed to sloughing of the necrotic tissue around the penetrations. After 3 weeks, the dermal penetrations began to close, a glassy covering and pigmentation were evident on the wound surface and petechial hemorrhaging and erythema had diminished. Thus the first

gross signs of healing occurred after 3 weeks. Regeneration of scales was evident on the wound perimeter at weeks 5 through 6 with expansion of the scale cover and pigmentation of the wound area through week 13. In the wound center, only a slight, grey colored indentation was present in weeks 8 through 13.

Lethal Wounds

The wounds described here were from fish killed by sea lamprey in less than 36 hours and up to 8 days - with most of the fish exhibiting a single wound. The gills on most of these fish were pale thus suggesting anemia.

The central portion of the wound, where the lamprey tongue penetrated the dermis, had an average diameter of 4.5 mm and exposed pale ulcerated necrotic muscle which was at times hemorrhagic. One fish, which had two wounds, had a large dermal penetration in one of the wounds which measured 9.0 by 24.0 mm across.

The peripheral portion of the wound area was lacking scales and ranged from a white abraded area to an area which had greyish-black to greyish-brown pigmentation present and was at times hemorrhagic. One of the wounds had many small perforations through the dermis to the muscle (Figure 16).

One fish survived an 8 day lamprey attachment but was near death 10 days following detachment. The sea lamprey shifted its position slightly thus causing a larger wound area, and at lamprey detachment, three dermal penetrations to the muscle were evident (Figure 17). Two of the dermal

openings were about 4.0 mm in diameter while the other was 2.0 mm in diameter. By day 4, following lamprey detachment, a fungal infection was present over the wound area and at day 5 an area of edema surrounded the wound. At week 1, two of the dermal openings were no longer separated but were now part of a larger dermal opening which at its maximum dimensions measured 24.0 by 10.0 mm across, while the other dermal opening measured 5.0 by 6.0 mm across (Figure 18). The muscle was ulcerated and hemorrhagic in these areas. The peripheral portion of the wound area was bleached white and was in the process of sloughing. The wound perimeter had a brown rim with hemorrhage and erythema evident in that area. At 10 days the dermal openings had enlarged another 2.0 mm (Figure 19). The hemorrhagic appearance of the wound suggested possible bacterial infiltration.

Many of the lethal wounds did not appear to be any more severe than the wounds of other fish that had survived sea lamprey attacks. The mortally wounded fish frequently had pale gills which suggested anemia caused by a substantial blood loss.

Lennon (1954) observed 1,189 sea lamprey wounds on 18 species of fish from Lake Huron and found that 22.2% were healed or in the process of healing and 77.8% were new wounds. Most of the wounds were on white suckers and of these 20.3% were healed or in the process of healing while 79.7% were new marks. He thought that most of the white suckers attacked by lampreys die as a direct or indirect

covering the entire range of human experience, catalogued by speaker and subject, all items can still be retrieved within minutes.

Figure 16. Rainbow trout killed within six days by a sea lamprey - note perforations through dermis

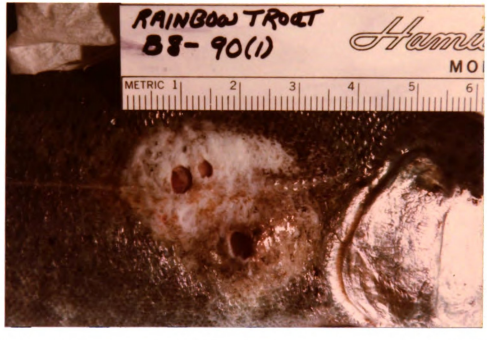

Figure 17. Eight day sea lamprey wound on a rainbow trout

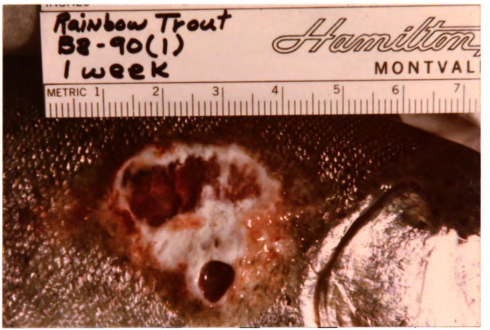

Figure 18. Eight day sea lamprey wound one week after detachment - note enlarged hemorrhagic wound area

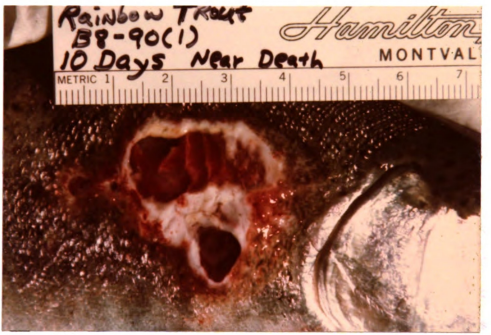

Figure 19. Eight day sea lamprey wound ten days after detachment - note enlarged hemorrhagic wound area

result of the attacks which would account for the larger percentage of newer wounds than older wounds. Fish not fatally wounded by lampreys may succumb to secondary fungal infections. He found that the fungi Saprolegnia parasitica and Leptomitus lacteus were the only common secondary invaders on lamprey wounds on fishes, with Leptomitus lacteus occurring more often on fish from Lake Huron and those in the aquaria.

Parker and Lennon (1956), in laboratory experiments, found that a small number of rainbow trout and white suckers which survived sea lamprey attacks died soon after from fungus infections in their wounds or from turbid water conditions. Seven rainbow trout which survived attacks of 5 to 39 hours duration recovered and their wounds healed completely.

Histopathology

A section of normal fish integument is shown in Figure 20 for comparison to the changes that occur as a result of a sea lamprey attack.

Wound Development

Four Hour Wound: The epithelial elements were essentially absent through much of the wound area on most fish and that which was present varied from a ragged appearance to nests of necrotic basophilic material which at times was dispersed with mucous cells (Figure 21). A residual layer of basal cells was at times present near the wound edge

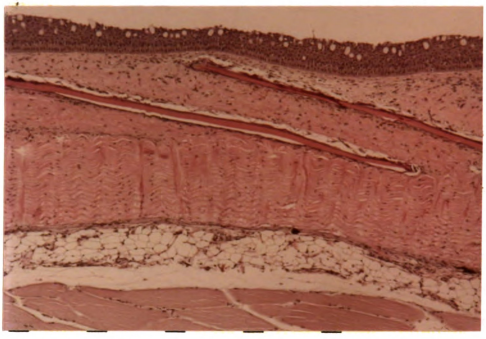

Figure 20. Normal integument from a rainbow trout
H & E. X40.

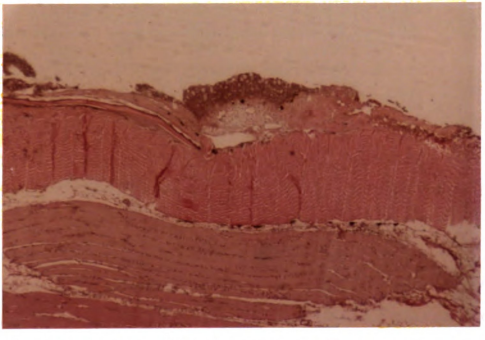

Figure 21. Wound area after four hours of sea lamprey attachment - epithelial elements are essentially absent
H & E. X25.

under the necrotic epidermis but most had lost their typical columnar form. Most of the remaining epidermis was edematous and many of the nuclei had taken up bizarre forms which included elongated spindle forms, condensed nuclear chromatin and karyolytic nuclei. Most of the wound area was lacking scales.

Much of the dermal stratum spongiosum had undergone caseation necrosis, was more basophilic, and contained many degenerated nuclei and fibrocyte remnants. The caseous nature of both the epidermis and stratum spongiosum at times made differentiation of these layers impossible. In some areas there was edematous separation and fragmentation of collagen fibers. The melanophores in the upper part of the stratum spongiosum throughout the wound area had undergone fragmentation and margination.

The dermal stratum compactum, hypodermis, and muscle all appeared normal.

Twelve Hour Wound: In addition to the epidermal pathology at 4 hours, the remaining epidermis at times exhibited caseation necrosis. A residual layer of basal cells which took on a vacuolated appearance was at times seen near the wound edge. Some areas of the wound were lacking scales and in some areas scale uplifting was evident (Figure 22).

Besides the changes of the dermal stratum spongiosum described for the 4 hour wound, some areas were vacuolated. Some of the melanophores in the wound area remained condensed while others had undergone fragmentation and margination.

In one case rod shaped bacteria covered the stratum spongiosum in one area while another case had evidence of hemorrhage on the surface of the wound area. One wound had a major penetration through the dermis and a blood clot was located in that area.

Damage in the dermal stratum compactum was generally confined to the upper area with the exception of one wound in which penetration of the dermis had occurred and caseation necrosis was present (Figure 23). The damage that occurred in the upper stratum compactum varied from a slight architectural disturbance to a limited caseation necrosis. At times slight edematous separation of collagen fibers occurred below this area resulting in a pale dermis. The hypodermis appeared normal except in one wound in which the hypodermis was disrupted where wound penetration occurred.

The longitudinal muscle below the damaged stratum compactum varied from normal to hemorrhagic. Some caseation necrosis had occurred in the area immediately below the dermal penetration in one wound.

Two Day Wound: Besides the epidermal lesions mentioned earlier, the epidermis at the wound edge had lost its organization and the cell layers were undergoing extensive necrosis. Basal cells were exhibiting cloudy swelling, and karyolysis within these cells was evident.

The dermal stratum spongiosum at this time was absent over parts of the wound surface, and that which remained demonstrated changes similar to those described earlier in the developing wound.

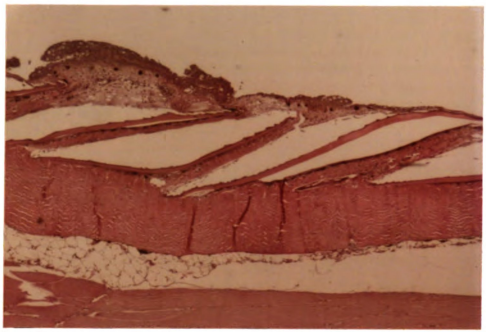

Figure 22. Twelve hour sea lamprey wound showing scale uplifting
H & E. X25.

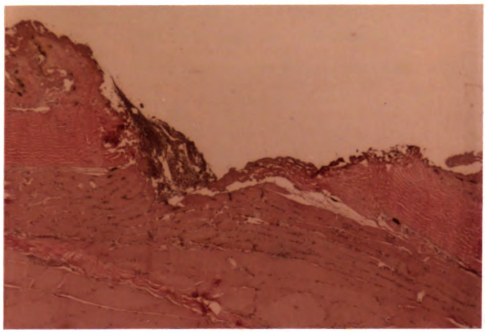

Figure 23. Twelve hour sea lamprey wound showing area of dermal penetration
H & E. X25.

The dermal stratum compactum had one major penetration in which caseation necrosis, accompanied by some fragmentation, had occurred along this immediate edge (Figure 24). The degree of architectural organization of the stratum compactum through the wound area had deteriorated and many degenerated nuclei and remnant of fibrocytes were evident. Edematous separation and some dissolution of collagen fibers had occurred which resulted in a pale appearance of the stratum compactum. Some surface areas of the stratum compactum were undergoing caseation necrosis while other areas had dermal fragmentation near the surface where the stratum spongiosum was absent. Disruption of part of the hypodermis and some cellular infiltration were evident.

Muscle fiber degeneration occurred in the proximity of the dermal break (Figure 25). Various stages of muscle necrosis were present accompanied by edematous separation of muscle fibers, a ground glass appearance of muscle fasciculi, sarcoplasmic dissolution, and caseation. Loss of striations had occurred in many of the necrotic muscle fibers along with karyolysis of sarcolemmal nuclei. Some cellular infiltration and hemorrhage had occurred in the upper muscle layers. One sample exhibited a pitted necrotic area which penetrated into the muscle below the dermal opening and the muscle in this region was pale in color.

Ten Day Wound: In addition to the previously described epidermal lesions, the epidermis at the wound edge exhibited a high degree of intercellular edema which had progressed to

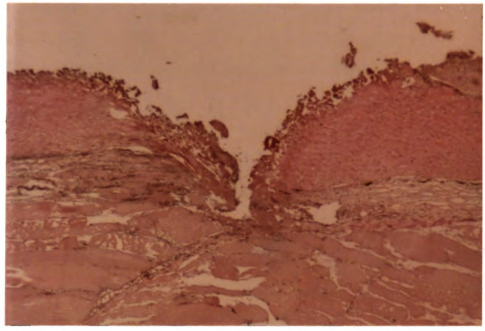

Figure 24. Two day sea lamprey wound showing area of dermal penetration with muscle necrosis below
H & E. X25.

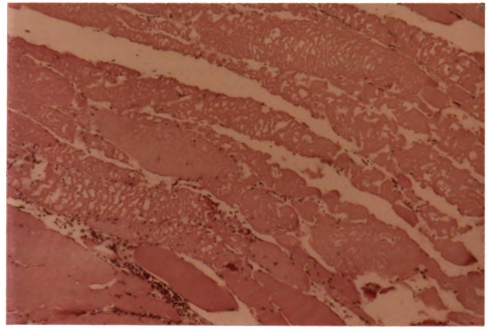

Figure 25. Muscle necrosis in a two day wound by a sea lamprey
H & E. X40.

the point where the epidermal elements had taken on a vacuolated appearance. Some of the epidermis was ragged and irregular in appearance with degenerated cells at all levels and the mucous cells were generally concentrated near the surface.

In one instance there was evidence of wound healing before the lamprey had detached and reepithelialization over the area of dermal penetration had occurred. The newly formed epidermis was thicker (0.37 mm) than that of the surrounding normal epithelium (0.22 mm). This thickening was attributed to the great amount of intercellular edema present. Many nuclei had taken on bizarre forms with condensed and karyolytic nuclear chromatin. Many of the basal cells exhibited cloudy swelling. Mucous cells were concentrated near the surface, and polymorphonuclear leucocyte infiltration occurred within the new epidermal tissue. The new epidermal tissue had also exhibited some concurrent dissolution. A trichrome stain of this sample demonstrated early stages of fibrogranulation through the dermis and into the upper muscle fasciculi where the lamprey tongue had earlier penetrated. From these phenomena it was concluded that the sea lamprey had fed early after first attacking this fish and then having discontinued feeding, hung on to the fish allowing for the healing process to occur.

In the 10 day wound samples the scales were absent through the wound area, however, some were uplifted and broken near the wound edge.

The dermal stratum spongiosum was partially to completely absent throughout the wound area. That which remained had changes similar to those described for earlier wound times. Cellular infiltration had occurred in parts of the stratum spongiosum with PMNs predominating. Other lesions of the stratum spongiosum included dissolution, and hyalinization and uplifting near the wound edge.

The dermal stratum compactum was absent throughout a large portion of the wound area which resulted in exposed muscle. In addition to previously mentioned lesions, many areas of the stratum compactum exhibited fragmentation of collagen fibers and the periodicity of collagen fibers was diminished in several areas which resulted in less order of the planar arrangement. One wound had cellular infiltration within the stratum compactum while another had it occurring above and below the stratum compactum. Hemorrhage and cellular infiltration, with PMN predominance, occurred at times within the hypodermis and one sample had congested blood vessels in this area.

Degeneration of muscle fibers occurred in the area of the dermal opening and the various stages of muscle necrosis present in the 2 day wound were also present here but more widespread. Myophagia was evident in some areas of the necrotic muscle and hemorrhage, resulting from the destruction of capillaries, was present in the area of muscle damage. Cellular infiltration occurred with heavy PMN predominance in one wound. Some of the samples had a large area of muscle pitted out below the dermal opening.

The histopathological observations made of the wound development gave an excellent picture of the type of damage a sea lamprey inflicts on a fish. After 4 hours of attachment, most of the epidermis and scales were destroyed in the wound area and damage was present all the way through the stratum spongiosum. At 12 hours, damage in the stratum compactum had occurred in the upper area, and in one wound, actual penetration had occurred with some local muscle necrosis evident below this. One fish, after a 12 hour attachment, had some rod shaped bacteria over the stratum spongiosum. After 2 days the lamprey tongue had penetrated through the dermis and had inflicted muscle damage to the host fish. After 10 days of lamprey attachment the stratum spongiosum was completely destroyed in some fish and the stratum compactum had a large area of penetration which resulted in more muscle exposure. Although some cellular infiltration and hemorrhage occurred within the muscle of the wound caused by a 2 day lamprey attachment, these changes were more pronounced in the 10 day wound. The cellular infiltration was present from the stratum spongiosum all the way down into the damaged muscle. Some samples showed predominance of PMNs, and myophagia was also evident.

Lennon (1954) observed that the fish skin is affected even by a temporary attachment of sea lamprey; incomplete attachments result in interruptions in the mucous coat and scales, and scoring of the skin. He found that damage to muscle tissues often appeared greater than could have been

caused by the suction action and rasping tongue. Besides mechanical injury, he demonstrated a cytolytic or histotoxic property in the secretions of the sea lampreys' buccal glands by injecting small amounts of these secretions subcutaneously in living fish. Reactions were produced in rainbow trout, brown trout, brook trout, white suckers and longnose suckers with death resulting in certain specimens. The integument appeared to be the most resistant while the muscle and vascular tissue the least resistant. In the present study the stratum compactum of the integument was also the most resistant and was only penetrated by the mechanical injury induced by the rasping tongue of the lamprey. The resistance of this part of the dermis could be attributed to its layers of collagen. Once the dermis was penetrated, muscle necrosis began to encompass a larger area. Muscle necrosis occurred away from the penetration area thus indicating the histolytic actions of the buccal gland secretions.

Many of the changes described for the developing wound were the result of some mechanical damage inflicted by the feeding lamprey. Damage is also inevitably caused by the digestive-like secretions from the lamprey. Damage may also have resulted from water entering the wound area and creating an osmotic imbalance and distortion of the tissue.

Much of the epidermal pathology that was evident in the present study was presumably caused by the buccal secretions of the sea lamprey. Roberts and Bullock (1976) noted that

the development of intercellular edema and the separation of epidermal malpighian cells by fluid from the dermis were the earliest indications of an inflammatory response in the epidermis. The formation of bizarre globules of basophilic nucleic acid throughout the affected area was caused by disordered epidermal metabolism which caused necrosis of the malpighian cells. They observed karyolytic changes of the cells and ultimately a complete dissolution of the cytoplasm and rupture of the nuclear membrane occurred. It was determined that any agent interfering with the metabolism of the epidermis resulted in this type of acute inflammation but it was most commonly caused by toxins produced by gram-negative microorganisms.

The stratum spongiosum is a complex upper layer of the dermis which includes the scales, pigment cells, mast cells and fine fibers which bind the epidermis. Disruption of this layer has detrimental effects on the overlying epidermis (Roberts and Bullock, 1976). They also described the stratum compactum as a dense layer of collagen penetrated by nerves and blood vessels which serve the stratum spongiosum. The hypodermis, which is a relatively loose tissue with a rich supply of blood vessels is probably the region where the lamprey first begins to obtain a rich blood supply for nourishment.

In wounds that were created by sea lamprey, the stratum spongiosum underwent caseation necrosis and was stained a basophilic color. Roberts and Bullock (1976) found that

integument which had been ulcerated for any length of time developed hyalinized superficial dermal collagen bundles, had a bluish sheen with loss of fibroblast nuclei demonstrated in H & E stained sections. This was known as the "water effect," and they suggested that it probably results from an alteration in the ionic charge on collagen which had been disrupted after exposure to water. They found that this layer of collagen is sequestered by macrophages after reepithelialization before reestablishment of the stratum spongiosum.

Roberts et al. (1973b) observed long strands of basophilic nuclear debris along the external surface of sinuses caused by identification tags on salmon and reported it to be caused by water damage to unprotected tissue.

The inflammatory response in the lamprey wound was evident after a 10 day attack, but some signs were evident as early as two days. Many of the cells were identified as PMNs; others were not so easily identified but because of their abundant cytoplasm and unlobed nuclei they were assumed to be macrophages. An early infiltration of neutrophils in injured fish tissue was reported by Ellis (1977). A neutrophil response has also been described for muscle injury caused by tagging salmon parr (Roberts et al., 1973a), aeromonad infections in brown trout (Thorpe and Roberts, 1972), and infections caused by lernaied copepods in white bass, Morone chrysops (Joy and Jones, 1973). Finn and Nielson (1971a, 1971b) found that neutrophilia occurred in 24 hours

after intraperitoneal injection of adjuvant in rainbow trout and lasted for less than a day, while Hines and Spira (1973) demonstrated that experimental infection of the mirror carp, Cyprinus carpio, with Ichthyophthirius multifiliis caused neutrophilia to occur within 48 hours and lasted about 8 days.

Roberts and Bullock (1976) reported that the polymorphonuclear leucocytes play a minor role and that the predominant cell, even in acute inflammatory responses, was the macrophage which could be of tissue or monocyte origin. They also reported that macrophages were present in inflammatory foci in early stages of their development and that they are very phagocytic toward damaged melanophores of the stratum spongiosum.

Review of Literature on Wound Healing and the Inflammatory Response in Fish

Research of the wound healing and inflammatory response in fish is relatively recent. The following is a brief review of some of the work that has been done in this area.

Bullock et al. (1978), doing autoradiographic studies on superficially wounded plaice skin, found that wound coverage was rapid even at low temperatures and that this coverage was due to migration of malpighian cells from the periphery and was not accompanied by mitotic division of cells. Mittal and Munshi (1974) demonstrated that malpighian cell migration was the chief factor in epithelial wound closure. However, they did not relate their findings to temperature.

Mawdesley-Thomas and Bucke (1973) found that epithelialization took place within the first month following injury to goldfish with a No. 14 hypodermic needle. Capillary congestion and hemorrhage resulted in edema between the dermis and epidermis and there was an increase in chronic cellular infiltration. Increased fibroblastic activity with a proliferation of the collagen fibers occurred within the dermis. Areas around degenerating muscle had a pronounced increase in connective tissue elements and capillary proliferation was marked. Fibroblastic activity was greatly increased in this area and the cellular response contained both acute and chronic inflammatory cells with the chronic type predominating. They found that the amount of muscle damage was greater than anticipated and 28 days following injury most of the affected fibers had almost completely degenerated and there was little attempt at regeneration.

Roberts et al. (1971) studied the process of healing in skin lesions of fish suffering from ulcerative dermal necrosis. The dermal tissue became covered by tongues of epithelial cells extending in from the periphery if the fish were treated with malachite green to prevent fungus infection. Dermal melanocytes usually followed the epithelial cells into the center of the lesion. The epithelial cells divided rapidly after the lesion was covered by a single layer of cells. The cells produced were disorganized and took considerably longer to attain normal architectural organization with its complement of mucous cells. They found that the

rate of healing varied according to water temperature. In the summer healing was complete within a few weeks and in the coldest part of the winter no healing took place at all.

Laird et al. (1975) observed the effects of freeze branding on salmon. After 24 hours, a layer of flattened malpighian cells covered the initial brand area and no mucous cells were present in the healed epidermis at this time. At the edge of the regenerated epithelium there was a slight increase in mitotic figures. Severe muscle damage had occurred. The polymorphonuclear leucocyte was the predominant inflammatory cell after 48 hours although some myophagic histiocytes were present. It was found that intermyotomal fascia limited the spread of necrosis to adjacent myotomes and also provided a vascular supply to the muscle. The epidermis increased to its normal level of thickness by the seventh day but the organization was lacking. An increase in melanin cells was present beneath the epidermis and in the hypodermis. Damaged muscle was nearly entirely sequestered and myophagic monocytes were present but PMNs were absent. Myotubes, which stained a glossy blue-red, were also seen in small numbers. Fibroblastic activity appeared to originate from the fascial sheaths or hypodermis and move in toward the lesion center. By 14 days the epidermis appeared normal and had its normal complement of mucous cells and scale regeneration was evident at this time. Prominent myotubules and juvenile bundles occurred within the muscle. By the third week scale regeneration was

prominent except directly below the site of branding and muscle regeneration was almost complete. Melanin-containing cells appeared later within healing muscle fibrous tissue.

Roberts et al. (1973a), studying the histopathology of salmon tagging at a mean water temperature of 12°C, found a cellular response comprised chiefly of macrophages with a few polymorphonuclear leucocytes which occurred within 2 days in the hypodermis and around blood vessels. The cellular response increased in the hypodermal plane and continued up through the disrupted stratum compactum and down between the muscle bundles along the fascial planes and perimysia by the 8th day. Fibrogranulation tissue was present by 14 days. In the early lesion the dermal melanophores appeared as powdery brown granules which were ultimately phagocytized by monocytes in the fibrogranulation tissue. Polymorphonuclear leucocytes infiltrated the muscle within 2 days, and by 2-1/2 days myophagic histocytes were active around the sarcoplasm of lysed myotome fibers. Fibrogranulation surrounded the affected muscle by 7 days. The severely damaged muscle was completely replaced by fibrogranulation tissue by the 32nd day and the less severely affected muscle showed evidence of basophilic sarcolemmal tube formation and restriction thus suggesting regeneration. The perimysium and inter-myotomal fascial planes limited the extension of the muscle lesion. The long-term response in the muscle was that replacement fibrosis was dominant and that healing and regeneration of muscle fibers were not readily noticeable.

Roberts et al. (1973a), studying the tissue response of salmon tagging at different temperatures, found that the lesion in the fish held at 8°C was similar to that of fish kept at 14°C. The rate of development of the various cellular and tissue components was the major difference. Fibrogranulation tissue, which was well established in 12°C fish at 6 days, was not well developed until the 18th day in 8°C fish but was not as dense as that at the higher temperature. Fish held at a mean temperature of 4°C had a qualitatively different reaction from fish of the higher temperature. The muscle damage with hemorrhage was similar to those of higher temperatures and smaller numbers of PMNs in vessels adjacent to the lesion were present at 3 days. PMNs were not seen at the sites of muscle lesions. At higher temperatures the epithelial cells were hyperplastic at this time but at 4°C, pyknosis and increased mucous cells were considerable. During the following days a small number of PMNs and thrombocytes with a few macrophages appeared around necrotic muscle fibers. Monocytes with ingested melanin were also observed. These were not seen in any number until the 9th day. Myophagia was well established by the 12th day and PMN activity was considerable until the 15th day around the necrotic sarcoplasm. Regenerating fibers or sarcoplasmic budding were not seen at this temperature. At the 20th day fibrogranulation tissue was starting to grow into the necrotic areas and between adjacent myotomes from the hypodermal area. Its development was very light compared with the dense fibrous growth at 12°C at 12 days.

Anderson and Roberts (1975) compared the healing of minor surgical wounds in a temperate and a tropical teleost at various temperature ranges. The temperate fish used was the Atlantic salmon (Salmo salar) and the tropical fish was the White Mountain Cloud Minnow (Tanichthyes albonubes). There were significant differences in the rate of wound healing both within and between species at the lowest and highest temperatures. Temperature stress had little effect on the healing rates. The wounds healed at a similar rate as those reported for the healing of superficial skin wounds in man and other mammals even though the fish wounds were not just superficial but involved both integument and muscle. Few PMNs were seen in the wounds of either species. They were relatively more numerous at 5°C than at 23°C in the salmon but this difference was not observed in the White Mountain Cloud Minnow. In the salmon PMNs were not seen until 2 days at 5° and 23°C. Between 12 and 24 hours after the incision, macrophages were first seen within the perimysia surrounding the damaged muscle. Myophagia commenced at 24 hours at 23°c and 3 days at 5°C and was completed by 16 to 18 days at 23°C. Fibroblasts first appeared at 5 to 6 days in the White Mountain Cloud Minnow and the salmon at the temperature of 23°C. It appeared that the simple non-continuous trauma of a surgical incision was only a weak stimulus to fibroplasia. PMNs were seen in the muscle at 3 hours at 23°C in the White Mountain Cloud Minnow but not until 48 hours in the Atlantic salmon at this same

temperature. The perimysial fascial planes limited the spread of necrosis in the muscle. In both species sarcoplasmic regeneration was present within 4 to 7 days at warm temperatures and from 8 to 38 days at cooler temperatures. Early stages of myofibrillar regeneration in the White Mountain Cloud Minnow were present at 24 days at 10°C. Advanced muscle regeneration was seen in both fish within 4 weeks at the warmer temperature. Myofibrillar regeneration was present in the form of small, hyaline eosinophilic buds which, after 18 days, had developed into small muscle bundles with the fibers irregularly arranged. Regenerated fibers were distinguished by their size differences. Wounds were covered in less than 2 hours at warm temperatures and within 24 hours at cold temperatures. They pointed out that the rapid epidermal wound closure has a major survival advantage in limiting entry of fungal spores.

Mittal and Munshi (1974), not relating their findings to temperature, found that epithelialization occurred in 4 to 6 hours after a surgical incision in the skin of Rita rita. Exposed muscle bundles lying in the wound gap started disintegrating 2 hours after injury and were replaced by irregularly arranged fibroblasts and blood capillaries by 5 to 6 days. Myoblasts appeared at the level of the old muscle bundles and differentiated into muscle fibers at 7 to 8 days following injury. Wound repair was complete in 26 days without any sign of a scar left on the surface.

Roberts and Hill (1976) found that the healing process of brown trout recovering from ulcerative dermal necrosis was temperature dependent. At water temperatures of 12° to 14°C lesion healing was completed within 7 to 10 days and was observed to take 2 to 3 weeks or longer at water temperatures below 8°C. The healing epithelial covering was not stratified and contained few mucous cells. Usually stratum spongiosum melanophores were present around the edges of the lesion but no melanin layer in the dermis appeared at the center until resolution was complete.

Roberts et al. (1973b) observed that regenerating muscle fibers were numerous in chronic tag lesions. In H & E preparations these muscle fibers had a bluish hue and had large, active muscle fiber nuclei. Degenerating fibers showed myophagia. Muscle changes in the chronic tag lesion suggested active polymyositis. The slow rate of healing in such fish was demonstrated by the increased maturity of replacement collagen in the two sea winter fish (true salmon) compared with the one sea winter specimens.

Finn and Nielson (1971b) studied the effects of temperature on the inflammatory response in rainbow trout and found that a change in temperature after an insult altered the time of appearance of a certain occurrence and of its maximum development rather than the qualitative nature of the response. There was a delay in a response at cooler temperatures in most cases and it was determined that the PMN response was delayed from 1 day at 15°C to 4 days at 5°C.

It was judged from histological sections of injured tissue and differential leucocyte counts in tissue smears that there was little qualitative difference in the rainbow trout response to the injection of killed staphylococci or complete Freund adjuvant whether the fish were kept at 5° or at 15°C. Quantitative changes were evident and the appearance of macrophages within muscle fibers, the clearance of bacteria and necrotic tissue from the lesions, and the start of fibroplasia were delayed by as much as 50% in fish kept at 5°C compared with the responses in rainbow trout kept at 15°C.

Finn and Nielson (1971a) studied the effects of various injuries on rainbow trout and found that the inflammatory response was closely comparable with that of mammals although less intense and slower to appear and resolve. These investigators indicated that the perimysial fascial planes limit the spread of necrosis in muscle and act as a retaining barrier by limiting the spread of noxious agents, carry large vessels responsible for many infiltrating leucocytes and appear to be the source of fibrocytes and fibroblasts. No sarcoplasmic budding or muscle fiber regeneration were ever observed.

Wound Healing

Two Days: Most of the epithelial elements were absent over the wound area in 2 days and the elements which remained exhibited a high degree of spongiosis leaving only a vacuolated appearance of the epithelial elements with a high

degree of dissolution in some areas. Many of the nuclei took up bizarre forms with karyolytic and condensed nuclear chromatin. Mucous cells, when present, occupied the surface layer of the necrotic epidermis. A residual layer of basal cells was present in the epidermis which exhibited cloudy swelling, karyolytic nuclei, and condensed nuclear chromatin. Limited squames or ribbons of reepithelialization occurred in the outer wound area in some fish (Figure 26). One sample had PMN infiltration present in the remaining epidermis. Another wound sample had cloudy swelling of epidermal cells at all levels on the wound edge and most of the nuclei were swollen and had undergone karyolysis.

Reepithelialization was evident over two areas where dermal disruption occurred in one wound sample and it exhibited a high degree of spongiosis. Some of the newly formed epidermis was ragged, vacuolated in appearance, and the mucous cells occupied the upper one third (Figure 27). Both condensation and karyolysis of nuclei were evident and cellular infiltration was present with PMN predominance. The basal cell layers had exhibited cloudy swelling with most nuclei swollen and many had undergone karyolysis. This newly formed epidermis took on a thickness which ranged from 0.10 to 0.22 mm compared to normal epidermis outside the wound area which had a thickness of 0.09 mm. Intercellular edema and hyperplasia accounted for the extra thickness of the epidermis over the wound area.

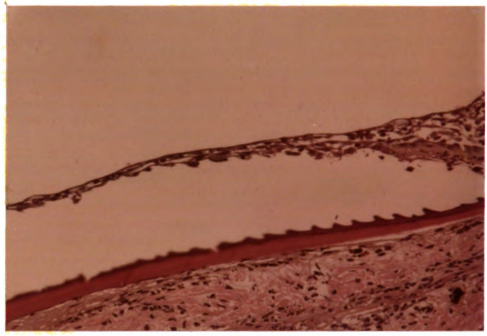

Figure 26. Reepithelialization after two days of wound healing
H & E. X100.

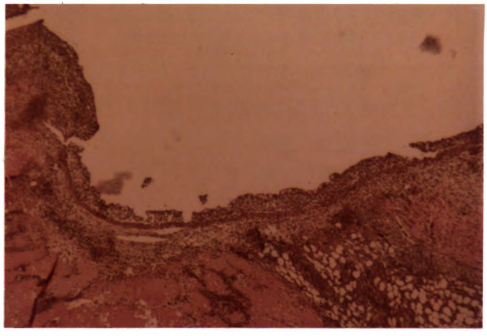

Figure 27. Reepithelialization over dermal penetration after two days of wound healing - note the concentration of mucous cells near the surface of the newly formed epidermis
H & E. X25.

The 2 day healed wounds varied from being completely absent of scales to having some present in the wound area.

The dermal stratum spongiosum ranged from being partially absent to almost completely absent throughout the wound area and much of what was present had undergone caseation necrosis and was more basophilic in color. Other areas were vacuolated in appearance while some areas were hyalinized. Degenerate nuclei and fibrocyte remnants were evident in the stratum spongiosum, and fragmentation and margination of melanophores ranged from a few to many in some areas. Heavy cellular infiltration was present which resulted in some phagocytosis of melanin from the melanophore fragmentation. At times deterioration of the stratum spongiosum and stratum compactum boundaries occurred making differentiation of these two layers in this zone almost impossible. One sample had some fungal and heavy bacterial invasion of the stratum spongiosum.

The dermal stratum compactum was absent in a large area where the wound pitted into the exposed muscle. The degree of architectural organization of the stratum compactum through the wound area was deteriorated and many degenerated nuclei and fibrocyte remnants were evident. Periodicity of the collagen fibers was diminished which resulted in less order in the planar arrangement. Edematous separation and dissolution of collagen fibers was evident which resulted in a more pale color of the stratum compactum. Some of the fibers were feather-like in appearance at the early stages

of dissolution. Caseation necrosis was evident in areas of the stratum compactum but was mainly predominant along the edge of the dermal penetration. Cellular infiltration varied from moderate to heavy throughout the stratum compactum. One sample had fragmentation and margination of melanophores in the exposed stratum compactum which was quite heavy in areas. This sample also had some bacterial invasion in the stratum compactum.

Cellular infiltration and vascular congestion ranged from moderate to heavy in the hypodermis in the wound area. One sample had heavy bacterial colonization in the hypodermal region of the dermal penetration.

A large area of muscle necrosis was present throughout the wound area. Various stages of muscle necrosis were present accompanied by edematous separation of muscle fibers, a ground glass appearance of muscle fasciculi, sarcoplasmic dissolution, and caseation. Much of the muscle lining the pitted wound area was completely necrotic and took on a vacuolated appearance. Hemorrhage and cellular infiltration with occasional heavy focal accumulations occurred in the damaged muscle. Edema fluid or sarcoplasm was evident between some of the muscle bundles and myophagia was present in some areas of the necrotic muscle. Containment of necrotic muscle occurred along fascial planes.

One sample had an area of necrotic muscle encapsulated below the area where the wound pitted into the muscle. Another sample had infiltration of rod shaped bacteria into

the necrotic muscle and phagocytosis of some of the bacteria was evident (Figure 28).

Early stages of fibrogranulation and fibrosis were evident in a trichrome stained sample which had reepithelialization over two areas of dermal penetration. Early stages of fibrogranulation penetrated through one of the dermal openings to the surface muscle layers.

Two Weeks: Reepithelialization occurred throughout the wound area and covered the area through the dermal opening over the necrotic muscle (Figure 29). Extensive spongiosis existed in the newly formed epidermis and mucous cells were concentrated on the surface. Swelling and karyolysis of nuclei was evident along with some nuclear condensation. The basal cells, which were irregularly arranged at times, were exhibiting cloudy swelling and many had lost their typical columnar shape. Swelling and karyolysis of nuclei was evident in these basal cells. The newly formed epidermis in the wound area had an average thickness of 0.23 mm compared to normal epidermis outside the wound area which had an average thickness of 0.16 mm. Two samples had epithelial cells just outside the dermal penetration area that exhibited cloudy swelling at all levels which had swollen and karyolytic nuclei.

Scales were absent throughout the wound area and some scale regeneration was evident near the wound edge.

The dermal stratum spongiosum ranged from being partially absent to almost completely absent throughout the

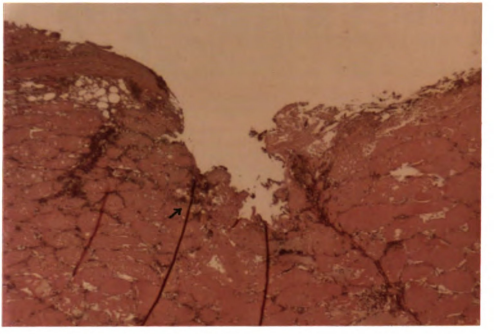

Figure 28. Bacterial infiltration into necrotic muscle two days following lamprey detachment - essentially no healing has occurred in the wound area
H & E. X25.

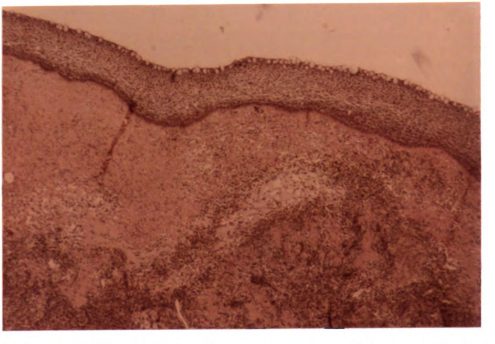

Figure 29. Reepithelialization over necrotic muscle after two weeks healing - note the concentration of mucous cells near the surface of the newly formed epidermis
H & E. X25.

wound area. The stratum spongiosum that was present had changes similar to those described for wound healing at 2 days. The stratum spongiosum exhibited some cellular infiltration and fibroblastic activity, and some areas had edemic separation of collagen fibers.

The dermal stratum compactum had a large area absent in the middle of the wound area except for one sample which had no dermal interruption. The stratum compactum that was present in the wound area had demonstrated changes similar to those described at 2 days wound healing. The stratum compactum exhibited some cellular infiltration and fibroblastic activity in the wound area and fragmentation of collagen fibers occurred at several points. One sample had two areas of the stratum compactum interrupted by areas of caseation necrosis.

The sample which had no interruption of the stratum compactum exhibited some caseation necrosis and dissolution of collagen fibers in a few isolated areas of the upper stratum compactum. The architectural organization of the stratum compactum in the wound area was diminished to a degree with edematous separation of collagen fibers and a slight loss of fiber periodicity evident. The stratum compactum in the wound area measured 0.49 mm compared to that outside the wound area which measured 0.32 mm.

Heavy fibroblastic activity and cellular infiltration were evident in the hypodermis, especially in the area near the dermal penetration, and fibrogranulation was present throughout this area.

A large area of muscle necrosis was present throughout the wound area except for the sample which had an intact stratum compactum; its muscle appeared normal. A caseation-type necrosis was evident throughout most of the damaged muscle with resultant loss of individual fascicular structures and muscle striations. Edema fluid or sarcoplasm was present in the necrotic muscle area. Heavy fibroblastic activity and cellular infiltration were evident. Fibrogranulation and neovascularization were exhibited in the area of muscle necrosis and myophagia was evident in some areas. Containment of fibrogranulation and necrotic muscle occurred along fascial planes (Figure 30). Muscle regeneration was evident in one sample in the form of many round basophilic buds of varying sizes with large nuclei (Figures 31 and 32).

Granulation tissue formation was present in the damaged muscle and extended under the stratum compactum into the hypodermis near the dermal penetration. A trichrome stain of one sample demonstrated that fibrosis was quite heavy in these areas. This trichrome stain also showed that many of the areas of the stratum spongiosum, stratum compactum, and muscle that exhibited caseation necrosis had fibrosis in these areas (Figure 33).

One Month: Epidermis was present over the wound area and it exhibited spongiosis which ranged from light to heavy but full architectural organization had not yet been fully attained by the epithelial cells. Many of the basal cells had undergone cloudy swelling and some were disorganized

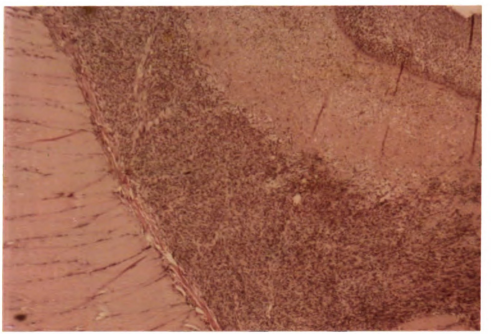

Figure 30. Fibrogranulation and muscle necrosis contained by muscle fascial plane after two weeks of wound healing
H & E. X25.

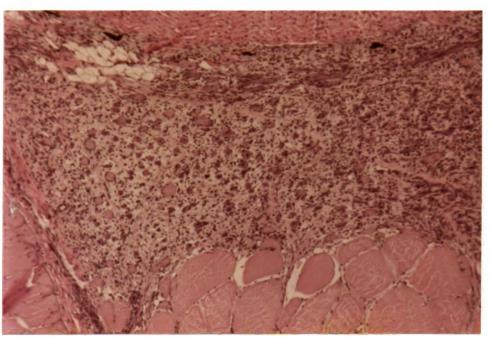

Figure 31. Muscle regeneration present after two weeks of wound healing
H & E. X40.

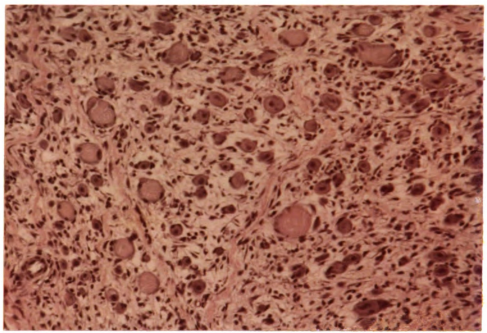

Figure 32. Muscle regeneration present after two weeks of wound healing
H & E. X100.

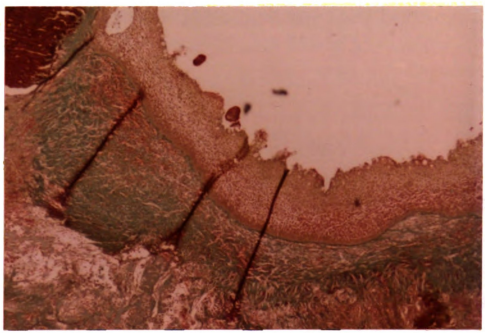

Figure 33. The trichrome stained section indicates fibrosis (green area) in the muscle area after two weeks of wound healing
Mallory's Trichrome. X25.

above the basement membrane. Swelling and karyolysis of nuclei were evident in both the basal cells and epithelial cells at other levels. Mucous cells were absent or lower in number in the wound center. The mucous cells that were present were usually in the top half of the epidermis. The epidermis in the wound center averaged 0.25 mm compared to that outside the wound area which averaged 0.17 mm. One area of very hyperplastic epidermis was present near the wound edge of one sample which measured 1.32 mm.

Scales were absent through most of the wound area. Some scale regeneration was present near the wound edge although one sample demonstrated some regeneration through the center of the wound (Figures 34 and 35).

The dermal stratum spongiosum ranged from being present to being absent closer to the wound center and that which was present had diminished architectural organization. Reorganization was evident from the very increased fibroblastic activity. Fragmentation and margination of chromatophores was evident in some samples while other samples failed to demonstrate this.

The dermal stratum compactum had an area interrupted by fibrogranulation which varied from small to large (Figure 36). The stratum compactum lacked full architectural organization and the periodicity of the fibers was diminished to a degree which resulted in less order in the planar arrangement. Edematous separation of the collagen fibers was evident especially near the wound center. Heavy fibroblastic activity

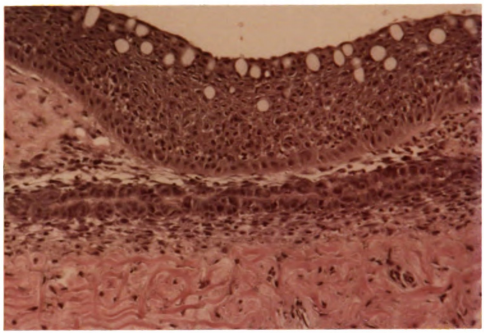

Figure 34. Scale regeneration after one month of wound healing
H & E. X100.

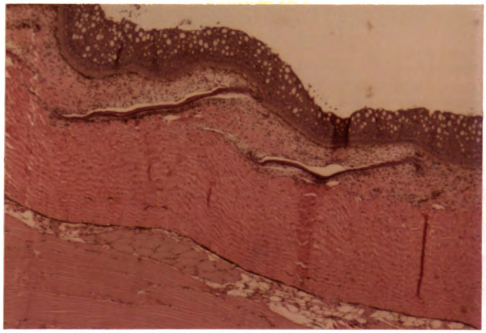

Figure 35. Scale regeneration after one month of wound healing
H & E. X25.

was present in the stratum compactum but was most predominant near the center of the wound area. Measurements taken of the stratum compactum of one sample had a thickness of 0.83 mm near the wound center compared to 0.37 mm outside the wound area. Some fibrogranulation was evident in the hypodermis near the wound center.

The muscle varied from normal in appearance to heavy fibrogranulation which encompassed the area of muscle damage. The muscle which exhibited the heavy fibrogranulation had heavy fibroblastic activity with some cellular infiltration. Neovascularization was evident throughout the granulation tissue and a scale or a piece of stratum compactum was at times seen trapped within the granulation tissue. Some melanophores with occasional fragmentation were seen throughout this area. The fibrogranulation was contained by the fascial planes.

One of the samples that exhibited fibrogranulation in the damaged muscle also demonstrated muscle regeneration (Figures 37 and 38). Regenerating muscle fibers had a blueish hue and had large muscle fiber nuclei. Many small muscle bundles with irregularly arranged fibers were evident.

A trichrome stain of two samples with heavy fibrogranulation in the area of muscle damage demonstrated fibrosis through the dermal interruption which extended down into the muscle and somewhat into the hypodermis on either side of the wound. The trichrome stain also showed some fibrosis in the stratum spongiosum.

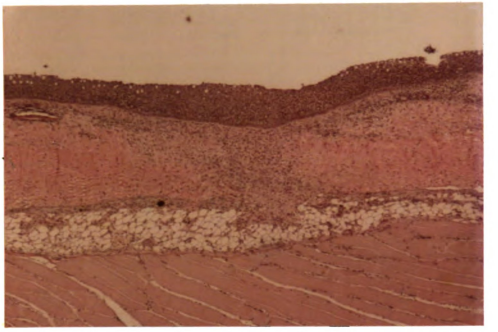

Figure 36. Wound area after one month of wound healing showing dermis interrupted by area of fibrogranulation where lamprey tongue penetrated earlier
H & E. X25.

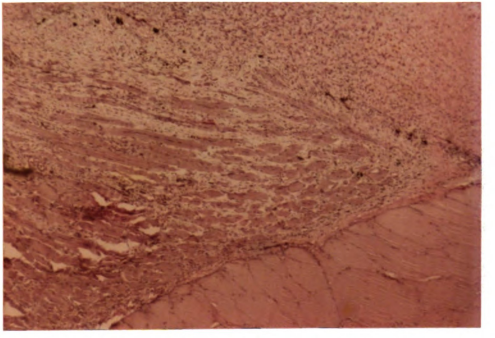

Figure 37. Muscle regeneration after one month of wound healing
H & E. X40.

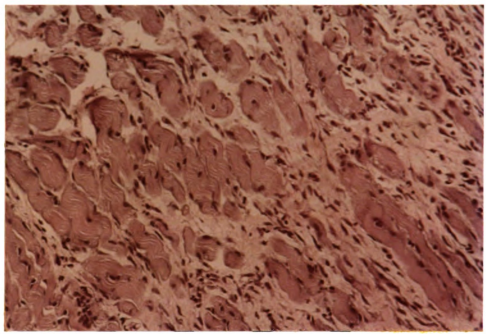

Figure 38. Muscle regeneration after one month of wound healing
H & E. X100.

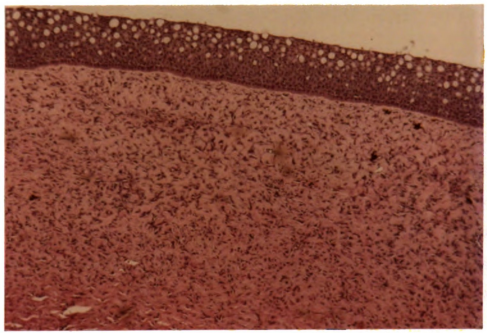

Figure 39. Wound area after three months of wound healing - epidermis was normal and dermal area showed fibroblastic activity
H & E. X40.

Three Months: Normal epidermis with its complement of mucous cells was present over the wound area (Figure 39), with the exception of one sample which demonstrated some cloudy swelling of the basal cells. Epidermis in the wound area measured 0.20 mm compared to 0.13 mm of the epidermis outside the wound area. One sample was entirely lacking epidermis except for a few isolated squames of necrotic and disorganized epithelium near the wound edge.

The scales were absent through most of the wound areas except for one sample where they were present, however, some scale regeneration was evident.

There was a wide variation in the changes of the dermal stratum spongiosum. One sample was normal in appearance while in others the stratum spongiosum was absent or somewhat disorganized. Some edematous separation and fragmentation of collagen fibers was evident.

Some of the samples exhibited fusion of the stratum spongiosum and stratum compactum throughout the wound area. Continued reorganization was evidenced by the fibroblastic activity which was present in the stratum spongiosum. Fragmentation and margination of melanophores was also exhibited.

The stratum compactum of the dermis was normal in one wound while another sample still showed an area interrupted by fibrogranulation, edematous separation of collagen fibers and some fibroblastic activity. The periodicity of collagen fibers was diminished in a few samples which resulted in less order of the planar arrangement. The presence of

fibroblastic activity was evidence of continued tissue reorganization (Figure 39).

The hypodermis was normal in all samples except one which had some fibrogranulation present where the stratum compactum was interrupted by granulation tissue.

The muscle was normal in all samples except in one which had limited fibrogranulation and neovascularization which occurred in the muscle immediately below the area of the stratum compactum that was interrupted by granulation tissue. Muscle fasciculi were disorganized in this area and most exhibited separation of muscle fibers.

The histopathology of the wound healing was studied on fish which had experienced an 8 day sea lamprey attachment. After 2 days of wound healing most of the epithelial elements were absent over the wound area. Limited squames or ribbons of reepithelialization occurred in the outer wound area in some fish. One fish did exhibit reepithelialization over two areas where dermal disruption occurred and intercellular edema and hyperplasia accounted for the extra thickness of the epidermis over this wound area. Most of the scales were absent throughout the wound area. The stratum spongiosum ranged from being partially to almost completely absent and that which was present had undergone caseation necrosis and was more basophilic in color. Phagocytosis of melanin from melanophore fragmentation was evidenced in the stratum spongiosum. One sample had some fungal and heavy bacterial invasion of the stratum spongiosum. The stratum compactum had

a large area absent where penetration was made by the lamprey tongue and below this was a pitted area into the exposed muscle. A large area of muscle necrosis was present throughout the wound area. In general, the cellular response was more pronounced following 2 days of wound healing. Only one sample showed early stages of fibrogranulation which occurred through a dermal penetration to the surface muscle layers. Relatively little evidence of healing was present after 2 days and this can be attributed to the large wound area which is going to take more time to resolve. There is also the possibility that buccal secretions were still present and active in the wound.

By 2 weeks reepithelialization had occurred throughout the wound area and covered the area across the dermal opening over the necrotic muscle. Extensive spongiosis occurred in the newly formed epidermis and mucous cells were concentrated on the surface. Some scale regeneration was evident near the wound edge. The stratum spongiosum ranged from being partially to almost completely absent throughout the wound area. That which was present had undergone caseation necrosis and was more basophilic in color. The stratum compactum had a large area absent in the middle of the wound area except for one sample which had no dermal interruption. As mentioned earlier the epidermis grew across this area so no muscle was exposed through the dermal interruption. A large area of muscle necrosis was present throughout the wound area and muscle regeneration was evident in one sample.

After 2 weeks wound closure had occurred thus the wound interior was closed off from the aquatic environment. Tissue resolution was in progress and fibrogranulation was evidenced throughout the wound area. No bacterial or fungal infections were associated with the wounds at this time.

At 1 month of healing the epidermis present over the wound area exhibited a spongiosis which ranged from light to heavy but full architectural organization had not been fully attained. Scale regeneration was evidenced at this time. The stratum spongiosum varied from being present to being absent closer to the wound center. The stratum compactum had an area interrupted by fibrogranulation where lamprey tongue penetration had once occurred. The muscle varied from normal in appearance to a heavy fibrogranulation encompassing the area of muscle damage. One sample demonstrated muscle regeneration at this time. After 1 month the wound area was still in the process of resolution as demonstrated by the fibroblastic activity throughout.

After 3 months, a normal epidermis with its complement of mucous cells was evident over the wound area except on one moribund fish sampled on day 85; its epidermis was entirely lacking. Scales, although absent through most of the wound area, had some regeneration. The stratum spongiosum ranged from normal in appearance to areas which were absent and other areas which were somewhat disorganized. The stratum compactum was interrupted by fibrogranulation in the central part of the wound on one fish. Muscle varied from

normal to some samples which exhibited edematous separation of muscle fibers in the area beneath the dermis. In general by 3 months of wound healing, most of the wound area was resolved but was not by any means completely back to normal as could be evidenced by the fibroblastic activity present throughout the wound area.

Phromsuthirak (1977) used electron microscopy to study the healing process of small surgical incisions in the skin of Gasterosteus aculeatus. He found that phagocytic epidermal cells migrated into the incision and joined at the center to close the wound. Maximum numbers of neutrophils occurred in the epidermis in the first 24 hours and then declined. Macrophages reached a maximum number on the 3rd day and returned to normal by the 8th day. The neutrophils were shown to be phagocytic but not as much as the macrophages. The incision was completely closed in 3 days. In the present study high numbers of neutrophils were noted in the newly formed epithelial tissue of one fish which had re-epithelialization during a 10 day lamprey attachment and in another fish whose wound healed for 2 days following an 8 day sea lamprey attachment.

During the sea lamprey attack on a fish the inflammatory response was not as pronounced as that following lamprey detachment. This may be due to the fact that the lamprey may have a suppressing effect upon cellular infiltration. McQueen et al. (1973) found that the metacercariae of Cryptocotyle lingua were well adapted to plaice as a second

intermediate host. Plaice had a high degree of tolerance which resulted in an almost negligible inflammatory response to the infection. However, Joy and Jones (1973) observed the inflammatory response within the dermis of white bass, Morone chrysops, infected with Lernea cruciata. The lesion initially resulted from the growth and expansion of the anchor processes of the copepod. Mild necrosis was present which was followed by a mild edema and neutrophil infiltration. Later macrophages appeared which resulted in phagocytosis of dead neutrophils and other cellular debris, and proliferation of fibroblasts, neovascularization, and maturation of fibroblasts to fibrocytes with collagen deposition occurred.

A shallow indentation remained even after 3 months of wound healing on the surface of the fish where the sea lamprey tongue at one time made its penetration through the dermis. During wound healing little exudate was present through the area where the lamprey's tongue had penetrated the dermis and epithelialization followed the contour of the wound. Ramachandran and Thangavelu (1969) found that the presence of a blood clot is not an important factor in the healing of wounds in Ophiocephalus sp. It was shown that fish exudate contains little blood and does not fill the entire wound cavity, so that in any extensive wound the scar and epithelialization usually fail to restore the original surface level.

Even though sea lamprey inflict a very large wound, if the fish is able to recover, wound resolution appears very good. In general, healing of the lamprey-inflicted wounds is similar to the changes of those described by other researchers on wound healing. The wound is closed by epithelialization by 2 weeks after lamprey detachment although the newly formed epidermis lacks the degree of organization seen in the normal. Some scale regeneration had commenced at 2 weeks after lamprey detachment and this type of regeneration was undoubtedly dependent on the condition of the stratum spongiosum, as this is where the scales originate. The stratum spongiosum at this time had shown early signs of fibroblastic activity thus indicating reorganization. Roberts and Bullock (1976) observed that damaged scales are usually sloughed through the epidermis and a new scale grows in if the germinal tissue is not damaged by the trauma.

Fibrous replacement in a lamprey wound was quite heavy at 2 weeks and at 1 month following lamprey detachment but some muscle regeneration was also evident. Finn and Nielson (1971a), Mawdesley-Thomas and Bucke (1973) and Roberts et al. (1973a) found that in damaged muscle, degenerative and fibrous replacement change were far more prominent than myofibrillar regeneration. By 3 months following lamprey detachment, the wound area was occupied by muscle with negligible fibrogranulation. Muscle regeneration in wounds of fish was confirmed by Ramachandran and Thangavelu (1969), Mittal and Munshi (1974) and Anderson and Roberts (1975).

In a wound inflicted by a sea lamprey, the perimysial fascial planes limited the spread of muscle necrosis which was also observed by many researchers studying wound healing in fish. The fibrogranulation in a lamprey wound appeared to originate from the perimysial fascial planes and hypodermis within 2 weeks following detachment.

Since wound healing is dependent on temperature, this has a direct effect on wound resolution of lamprey scars on fish. Lamprey-inflicted wounds take longer to heal during the winter months when the water temperature is low. Wounds inflicted on fish in Lake Superior would undoubtedly require longer healing times as the water is colder compared to the lower Great Lakes. Lennon (1954) observed that wound resolution of lamprey scars begins sooner in the summer than in the winter and attributed this to the higher metabolic rate of fishes in warm water.

Lethal Wounds

The histopathology of 3 lethal sea lamprey wounds inflicted on rainbow trout was studied. Two of these fish were near death after a lamprey attachment of 7 and 8 days. These two fish lacked epidermis throughout most of the wound area and scales were absent. A large area of the stratum spongiosum was absent and that which was present underwent caseation necrosis and was more basophilic in color. One of the fish had cellular infiltration in this area. A large area of the stratum compactum was absent resulting in exposed muscle. Bacterial invasion occurred through some areas

of the highly necrotic dermis of one fish and phagocytosis of bacteria was evident. Cellular infiltration occurred in the hypodermis of both fish and an extensive area of muscle degeneration was evident. Necrotic muscle was contained by the perimysial fascial planes and no appreciable hemorrhage was present. Their blood volume was quite low as indicated by a 2% hematocrit. The fish that had bacterial invasion in the dermis also had bacteria within the exposed surface muscle and enzymatic destruction of muscle was evident. Both fish had cellular infiltration within the damaged muscle and phagocytosis of bacteria was evident in the fish that was infected.

The third fish had experienced an 8 day lamprey attachment and was near death 10 days following lamprey detachment. This fish had a large open wounded area which became very hemorrhagic and infected with fungus after lamprey detachment. Histologically, much of the epidermis was absent over the wound area and poor quality reepithelialization from the wound edge was evident. Many PMNs were seen and scales were absent throughout the wound area. Much of the stratum spongiosum was absent and hemorrhage and cellular infiltration had occurred in this area. Most of the stratum compactum was absent which exposed muscle, and increased cellular infiltration was present in the stratum compactum that remained. Muscle necrosis was extensive and a large amount of hemorrhage and cellular infiltration was evident. Necrotic muscle was contained by the fascial planes. This fish had a

hematocrit of 15% at lamprey detachment and it fell to 10% at the time of sampling. This could be attributed to the large amount of hemorrhaging that occurred in the wound area. It is interesting to note that bacterial and fungal infections were not seen histologically. Although evidence of fungal infection was seen grossly at 4 days following lamprey detachment and the wound area became very hemorrhagic, thus indicating the possibility of a bacterial infection, none was seen histologically. The high amount of cellular infiltrate could have cleaned the wound area of any evidence of infection.

Defense Mechanisms Against Pathogens

A lamprey wound may be lethal in two ways. First the blood loss from the fish may be so intense that recovery is impossible; second, the fish under such a stressful situation with such a large wound is directly prone to infection. But of the 19.5% attacks which were lethal, most could be attributed to the high amount of blood lost by the fish. Infection seemed to play only a minor role in the wounds inflicted in this laboratory study. This may be attributed to the cleaner environmental conditions within the laboratory compared to those of a natural environmental setting. Only 9.5% of the fish in the wound development group showed any histological signs of infection and these were bacterial in nature and occurred in one fish within 12 hours of lamprey attachment. In the wound healing group of fish only 15% showed any signs of infection, and these were both bacterial

and fungal in nature and with most occurring within 2 days after lamprey detachment when the open wound was exposed to an aquatic environment. Many of these fish did not show any adverse signs of the infection. Since these were timed experiments, many of the fish that were infected may have succumbed to a full blown infection if they were allowed to continue instead of being sacrificed. Many of the lamprey attacks were terminated before significant anemia developed, and thus, these fish were able to cope better against infection than those fish which the lamprey were allowed to feed upon to satiation as most often occurs in nature.

Lennon (1954) sampled 1,189 complete wounds on 18 species of fish from Lake Huron and found that only 22.2% were healing or healed which indicated that a host subjected to an attack was usually killed. He also found that hosts which had incomplete wounds were susceptible to invasion by fungi at the attachment site.

Roberts et al. (1973c) found that tagging caused openings in the integument where opportunist pathogens and secondary infectious agents such as Saprolegnia or Myxobacteria can gain entry into the body.

Many factors are probably involved in preventing the infection of lamprey wounds. One of the most important factors is the rate of epidermal closure of the wound. The inflammatory cells play a role in the phagocytosis of pathogens that have entered the wound; phagocytosis was evident in those fish which had bacterial infections.

Since it would be detrimental to the sea lamprey if its host became infected during feeding, it may be suggested that the buccal gland secretions themselves may have properties which prevent the establishment of pathogens within the wound area. It is known that these secretions are lytic in nature and thus could very well have this role. Gibbs (1956) did a histochemical analysis of the two recognized constituents of the buccal gland secretion. One of the constituents was lipid droplets which had triglyceride-containing centers surrounded by phospholipid-rich shells. The other constituent was a colloid substance and was probably a muco- or glycoprotein or possibly a neutral mucopolysaccharide. Of the two identified constituents of the secretion, she suspected that the colloid substance was the hemolytic and cytolytic agent of the secretion. The lipid droplets were not thought to perform any of these functions although it was pointed out that lysolecthin, which is a phospholipid formed by lecithinase A of rattlesnake venom, is known to have powerful hemolytic action.

Mucous cells may also play a role against pathogens as many of the mucous cells in the newly formed epidermis were concentrated near the surface during the first two weeks of wound healing. Fletcher and Grant (1968, 1969) suggested that continuous early replacement of mucous cells within the healing epidermis prevents colonization by parasites, fungi and bacteria and they obtained evidence of more specific chemical and immuno-chemical reactions within the mucous of

plaice, Pleuronectes platessa. Baldo and Fletcher (1973), besides finding immunoglobulins in the mucins of plaice, found C-reactive proteins to also be present. C-reactive protein is a non-antibody precipitin that has a broad specificity for determinants commonly found in cell walls or surface structures of invading pathogens. They pointed out that other researchers have detected C-reactive proteins in serum and other serous fluids during pathological conditions in which tissue injury, inflammation, or carcinoma occurs in mammals and also in certain infections in man.

Melanin may have a protective role against pathogens. Fragmentation and margination of melanophores were exhibited in the stratum spongiosum of the developing wounds. This type of reaction was more limited after a 10 day sea lamprey attack which could be attributed to more damage and larger areas of the stratum spongiosum being absent. During wound healing melanophore fragmentation and margination were occasionally quite heavy and phagocytosis of melanin was evident after 2 days of wound healing. After 2 weeks of wound healing, some fragmentation of the melanophores was evident with limited phagocytosis of melanin. By 1 month some samples had fragmentation and margination of melanophores in the stratum spongiosum but it was also noted that melanophores were present in the fibrogranulation tissue of the resolving muscle. Melanophores were present at 3 months of wound healing in the stratum spongiosum although not in heavy concentrations.

Edelstein (1971) suggested that melanins in plants and animals may have a defensive role in that the free-radical characteristics of melanin and its precursers, and the ability of melanin to oxidize NADH to produce hydrogen peroxide, are both bactericidal properties similar to those which occur in polymorphs. These effects may operate during melanocyte migration over the surface of fresh wounds causing the destruction of surface bacteria before or during wound reepithelialization.

Roberts and Bullock (1976) found there is a progressive infiltration of melanin-containing cells into the healing mesenchymal elements of large skin wounds. This produces a very black lesion after one year. Roberts (1974) observed that, in certain ulcerative conditions, melanocytes grow into epithelialized scar tissue as it heals and develop into melanophores.

Blood Parameters of Wound Development and Wound Healing Fish

Lennon (1954) found that the feeding mechanism of sea lamprey is adapted for obtaining liquid food, the principal food being blood, sucked from the host fishes. Body juices enter the parasitic diet to a lesser degree and a considerable amount of reduced flesh, particularly muscle, is also ingested by the lamprey. Within the confines of the wound under lamprey attack, the capillaries are destroyed and blood and lymph are pumped from the wounds of fishes.

Gage and Gage-Day (1927) discovered that the buccal gland secretions of lamprey, when mixed with blood in sufficient quantity, inhibited clotting and had hemolytic properties.

Lennon (1954) found that the buccal gland secretions from sea lamprey prevented fish blood coagulation within certain time limits when the concentration of the secretion equaled or exceeded 25% of the total volume of the mixture.

Farmer et al. (1975) determined that the daily blood consumption by sea lamprey at 10±1°C ranged from 2.9 to 29.8% of their wet body weight per day with a mean value of 11.6±7.5% per day. This study was done on sea lamprey feeding on rainbow trout and lake trout. The lamprey were grouped into four weight classes, with the smallest weight class ranging from 16 to 50 g which conformed to most lamprey weights used in the present study. This smaller weight class had a mean blood consumption of 11.1±1.8% of its body weight per day.

Therefore, in the present research, one could assume that the daily blood consumption by the sea lamprey was 11.1% of its body weight per day. It was assumed that the rainbow trout had a blood volume equal to 5% of its body weight (Schiffmann and Fromm 1959; Conte et al. 1963; Smith 1966). If the average weight of the sea lamprey used during wound development was 13.5 g, it would consume 1.5 g of blood per day. The average weight of the trout used during wound development was 309.1 g of which 15.5 g was blood.

This means that each fish in this group lost approximately 9.6% of its blood per day. By using the regression equation, Farmer et al. (1975) described the relationship between blood loss of trout and time to death, e.g., log y = 3.311-1.533 log x, x is the amount of blood lost daily by trout, expressed as a percentage of its blood volume, and y is the time to its death in days. Using this equation, the fish attacked during wound development would take approximately 63 days to succumb. The actual time would most likely be shorter as the lamprey grow during attachment and thus become more lethal. Since the lamprey attachment was terminated within a 10 day maximum for wound development, no deaths were noted.

The average weight of the lamprey used in the part of the study to develop the wounds for wound healing was 25.3 g. Thus these lamprey consumed an estimated 2.8 g of blood per day. The average weight of the trout in which wounds were inflicted for wound healing was 328.4 g of which 16.4 g was blood. Each fish in this group thus lost approximately 17.0% of its blood per day. At this rate, death would be expected about 26 days after lamprey attachment or sooner because the lamprey is growing and consuming more blood daily. All lamprey attachments were ended at 8 days within this group thus most lamprey attacks were not fatal.

Farmer et al. (1975) found that trout died in less than 2 days if the daily blood loss was equivalent to its total blood volume and in 14 days if it lost the equivalent of 25% daily.

During lamprey attachment the hematocrit progressively increased from a control value of 24.1±1.8% to a value of 30.7±1.8% after 12 hours of attachment (Figure 40A). This significant rise in the hematocrit could be attributed to the stress response of the spleen contracting and the additional erythrocytes entering the circulation (Wedemeyer, 1970). Casillas and Smith (1977) also found hematocrits to increase after stress. The hematocrit was 27.9±1.4% at 10 days of lamprey attachment thus indicating the inability of the smaller size lamprey to induce anemia even after ten days of attachment.

Changes in the hemoglobin concentration closely paralelled the changes in the hematocrit (Figure 40C). The hemoglobin had a control value of 6.6±0.7 g /dl and continued to increase up to the 12 hour lamprey attachment when it had a value of 8.6±0.4 g/dl. This significant rise was consistent with the rise in the hematocrit indicating that the increased circulating erythrocytes had a full complement of hemoglobin. The hemoglobin then decreased to 7.8±0.4 g/dl after 10 days of lamprey attachment thus following in line with the hematocrit. There was no significant change in the red blood cell precursers during this wound development period (Figure 40E), indicating that the additional cells entering the circulation were mature cells from the spleen with a full complement of hemoglobin.

The fish in the wound healing group all experienced an 8 day sea lamprey attachment. The hematocrit at lamprey

detachment was 20.7±1.9% which dropped significantly to 14.0±3.5% upon 2 days post detachment (Figure 40B). This drop could be 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 the fish may cause the hematocrit to change in different ways. The hematocrit then increased to 19.8±1.3% at 1 week and continued to rise slightly over time, with some slight fluctuations, and reached a value of 25.7±1.4% at 3 months. The increased hematocrit could be attributed to the epidermis covering the wound area and thus stopping the influx of water which may have caused hemodilution.

The hemoglobin curve followed closely the hematocrit curve over the wound healing period (Figure 40D). The hemoglobin concentration at lamprey detachment was 6.0±0.5 g/dl and dropped significantly to 3.7±0.9 g/dl at 2 days. This drop was probably due to a hemodilution effect as suggested by the lowered hematocrit at this time. The hemoglobin then increased to 5.7±0.4 g/dl at 1 week and continued at a fluctuating plateau to 3 months when it had a value of 5.7±0.2 g/dl.

An accompanying RBC precurser response also could account for the rising hematocrit at 1 week and beyond (Figure 40F). The red blood cell precurser value at detachment was 5.3±0.9% and was significantly higher than the control value of 2.2±0.7%. The number of RBC precursers continued to rise significantly after lamprey detachment.

Figure 40. Hematocrit, hemoglobin and red blood cell precurser 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 precursers (%) in wound development fish.
(F) Red blood cell precursers (%) in wound healing fish.

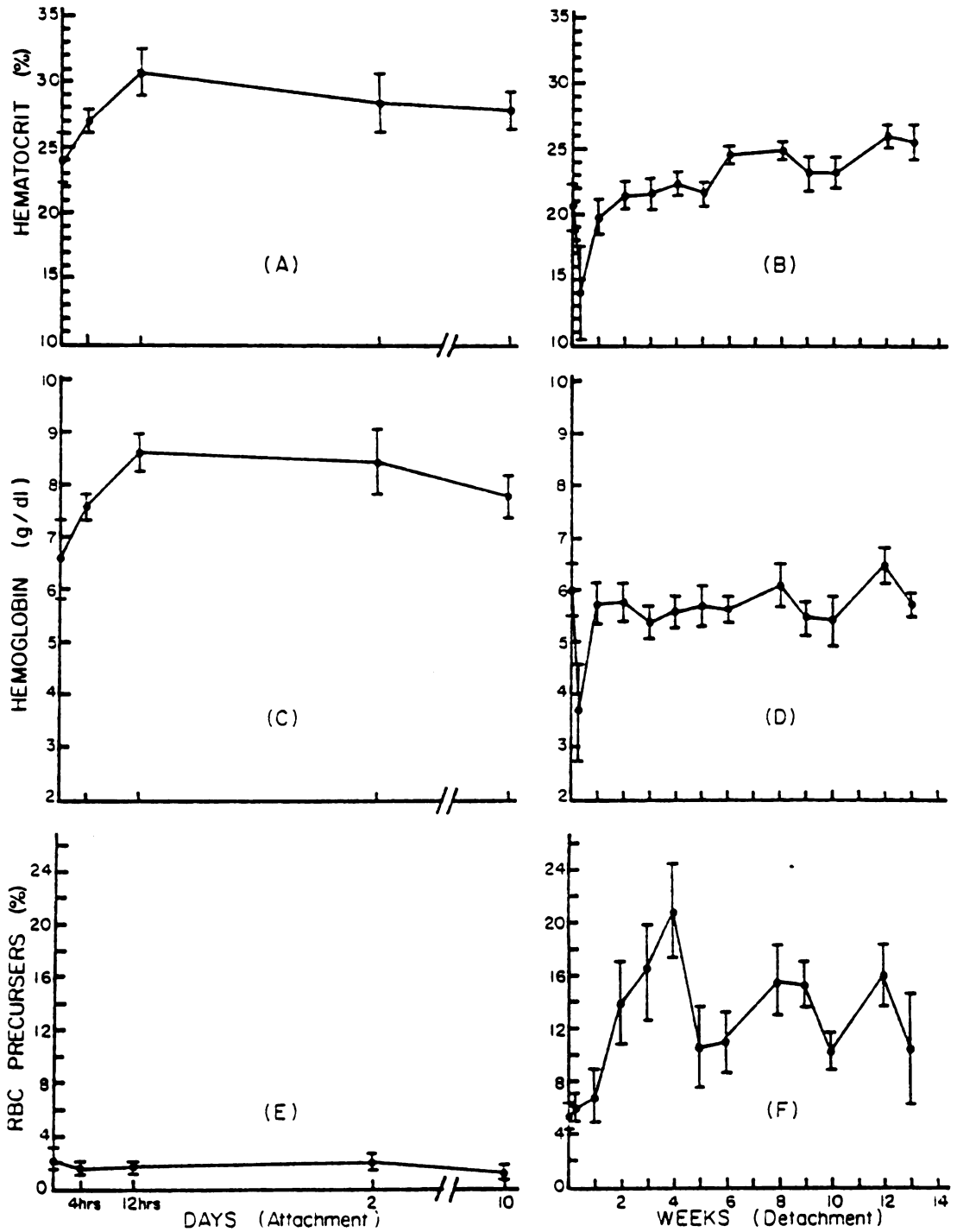
HEMATOCRIT (%)
(A)
(B)
HEMOGLOBIN (g/dl)
(C)
(D)
RBC PRECURSERS (%)
(E)
(F)
4hrs
12hrs
DAYS (Attachment)
WEEKS (Detachment)

Figure 40.

By week 2 the value was 13.9±3.1% and by week 4 the value reached a peak of 20.9±3.5%. By week 5 the number of RBC precursers dropped to 10.6±3.0% and maintained a highly fluctuating level to 3 months when the value was 10.4±4.2%. One would expect that the increased number of RBC precursers during wound healing would result in a lower hemoglobin concentration because of the reduced amount of hemoglobin carried by these immature cells. Although the hemoglobin concentration fluctuated around a value of 5.7 g/dl from 1 week through 3 months of wound healing, it was somewhat lower than the hemoglobin concentration of 6.6 g/dl in fish that did not experience a lamprey attack (Figure 40C and D).

The RBC precurser response tends to agree with Walker (1972), who found that after withdrawing 20 to 30% of the whole blood volume from rainbow trout the reticulocytes appeared as early as 5 days later and a high reticulocyte count of 40.44% remained at 20 days. He concluded that the reticulocyte increase appearing in the peripheral circulation is a long-term response for rainbow trout since the high reticulocyte count obtained on day 20 indicated that the response was still in progress.

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 that the first signs of significant hematocrit recovery came at 16 days following the last bleeding

and the hemoglobin concentration did not recover until 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. He reported that the reticulocytes rose from 2.99% at the last bleeding to 17.64% 11 days later. At day 30 neither the hematocrit nor hemoglobin values were significantly different from the controls and the reticulocyte percentages of the bled fish had declined to 5% and recovery was believed to be complete.

Smith et al. (1971) injected chinook salmon with phenylhydrazine, a hemolytic agent, and observed a severe anemia. It required 95 days for recovery at which time the hematocrit had returned to normal but the hemoglobin concentration was still below normal.

Yoffey (1929), working with dogfish, Scylliorhinus canicula, demonstrated that, after withdrawing 37 to 50% of the total blood, new blood formation begins to be evident about the 6th day and reaches a maximum about the 20th day.

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 and rainbow trout, they learned that the leucocrit and leucocyte-thrombocyte counts for both species were

depressed from control values after 96 hours of exposure to stressful conditions. Even shorter exposure times were found to elicit a response in the rainbow trout.

During wound development the leucocrit value had slight drops from the control value of 0.97±0.10% to 0.89±0.04% and 0.84±0.13% at 12 hours and 10 day attachments respectively (Figure 41A). These decreases in the leucocrit were not significant. One would expect sea lamprey attachment to cause a more significant decrease in the leucocrit from the control value. But the differential leucocyte count indicated that the lymphocytes decreased during lamprey attachment which is a typical stress response. The lymphocyte control value was 95.6±1.3% and then dropped significantly to 91.4±1.0% and 79.4±6.1% at the 4 and 12 hour attachment periods, respectively, before rising to 82.2±7.5% at the 2 day lamprey attachment (Figure 41C). 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 the leucocrit may not have dropped significantly during this time. It should be pointed out here that the differential leucocyte counts are relative percentages and in no way do they tell us the absolute amount that is present in the circulating blood.

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

Figure 41. Leucocrit, lymphocyte, and thrombocyte values during wound development and wound healing in lamprey-attacked fish.

(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.

* The blood smears obtained at ten day lamprey attachment were of poor quality, therefore a white blood cell differential was not obtained.

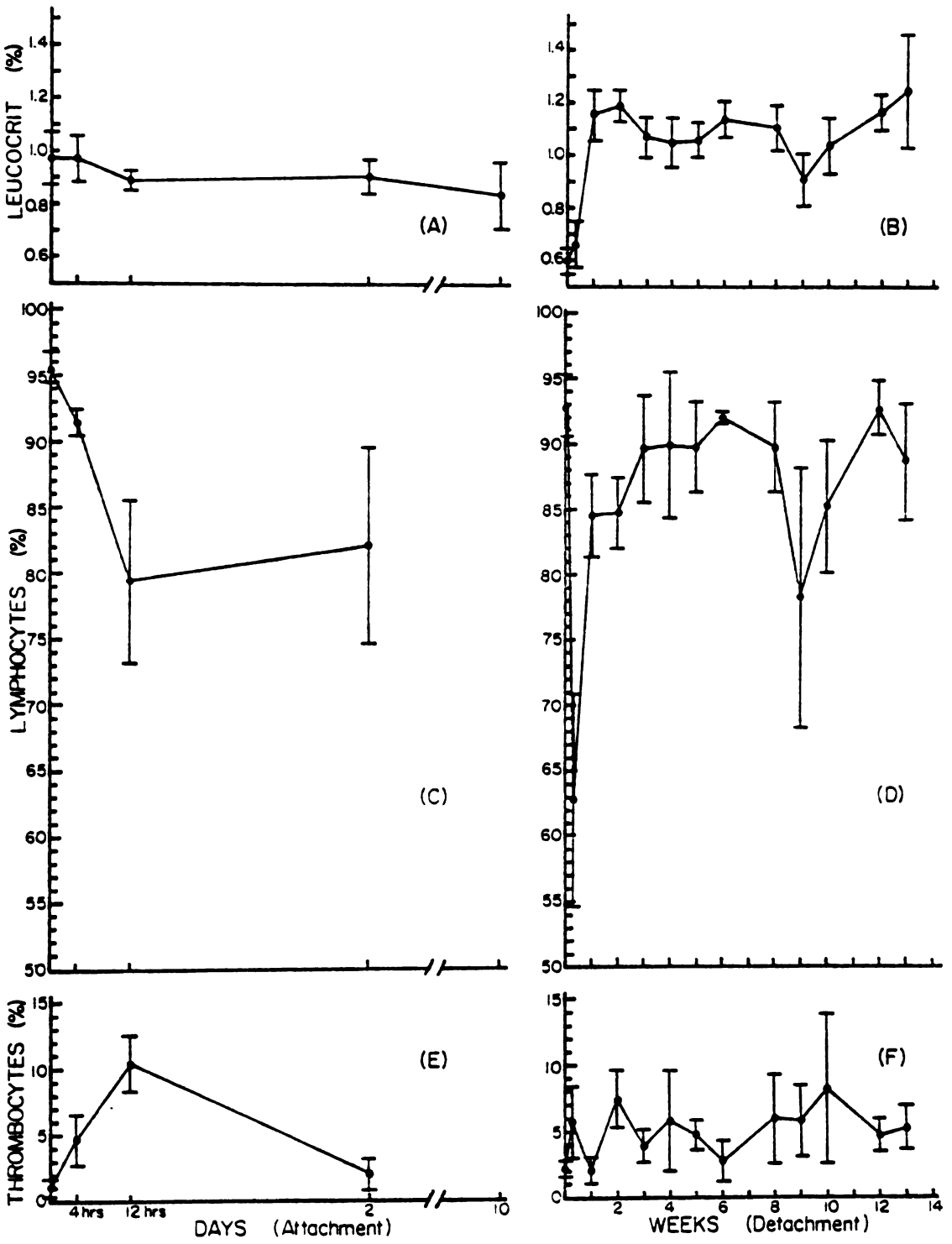
LEUCOCRIT (%)
1.4
1.2
1.0
0.8
0.6
(A)
(B)
LYMPHOCYTES (%)
100
95
90
85
80
75
70
65
60
55
50
(C)
(D)
THROMBOCYTES (%)
15
10
5
0
(E)
(F)
4 hrs
12 hrs
2
10
DAYS (Attachment)
2
4
6
8
10
12
14
WEEKS (Detachment)

Figure 41

immunological responsiveness in mammals. Evidence is now present showing that stress causes increases in circulating adrenal corticosteroid levels in teleostean fishes (Hane et al., 1966; Fagerlund, 1967; Wedemeyer, 1969; 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).

Weinreb (1958) found lymphopenia and thrombocytopenia in rainbow trout resulted from stress, cortisone, and ACTH, while heterophilia occurred after stress and ACTH injection. It was reported that exogenous ACTH produced an 80% reduction of circulating lymphocytes within 24 hours following administration. Since significant heterophilia did not result from cortisone, it was thought that the granulocyte forming centers were not under the adrenal cortical control as suggested for lymphocytes and thrombocytes. It was therefore suggested that multiple controls exist in the same hemopoietic organs where granulocyte and agranulocyte forming tissues are located.

Slicher (1961) demonstrated that neutrophilia is almost always associated with concomitant lymphopenia as was seen in the killfish, Fundulus heteroclitus, after cold shock, an injection of saline or ACTH.

Belova (1965), working with stress on Pink salmon, found that the decreased percentage of lymphocytes (24%) was accompanied by a corresponding rise in monocytes and

neutrophils. It was concluded that lymphopenia was a more reliable indicator of unfavorable environmental conditions and stress than was the erythrocyte count or any other hematological parameter.

Enomoto (1969) observed lymphopenia in Rudarius ercodes and other species resulting from cold shock which persisted for many days and was accompanied by total leucopenia.

McLeay (1973a) determined that ACTH injections resulted in marked decreases in the number of circulating lymphocytes and thrombocytes but an increase in neutrophils occurred which was relative because of decreased lymphocytes. As pointed out earlier, the increased neutrophilic series during wound development may have been relative but since the leucocrit did not decrease, it may have been an absolute increase. McLeay (1973b) found that all dosages of cortisol caused a marked decrease in the number of circulating small lymphocytes but not thrombocytes.

McLeay (1937c) studied the effects of a 12 hour and 25 day exposure of juvenile coho salmon to kraft mill effluent and found that the numbers of circulating small lymphocytes decreased greatly after 12 hours of exposure. After prolonged exposure the number of small lymphocytes returned to normal while the number of circulating neutrophils increased. It was concluded that the lymphocyte decrease was probably due to an increase in secretion of corticosteroids by the interrenal tissue caused by stress.

Hill and Fromm (1968) found that increased circulating levels of cortisol in rainbow trout resulting from chemical stress gradually returned to normal during chronic exposure. This would probably support the findings of McLeay and Gordon (1977) who concluded that leucocrit values are probably not a useful indicator of chronic conditions of stress in fish.

Bennett and Gaudio Neville (1975) showed that significant lymphopenia and neutrophilia occurred in 1 to 2 hours following cold shock in goldfish. The counts were back to normal in 4 hours.

In the wound healing group of the current experiment, the leucocrit at detachment was 0.59±0.04% which was signifcantly lower than the control leucrocrit of 0.97±0.10% (Figure 41B). After detachment the leucocrit increased significantly to a value of 1.16±0.09% at 1 week and remained at a fluctuating plateau for nearly 3 months. This decreased leucocrit at detachment would tend to be caused by a decreased lymphocyte number but the lymphocytes at detachment had a value of 92.8±2.2% (Figure 41D). Although a relatively high lymphocyte percentage was present, this only represented a relative percentage and may actually have experienced an absolute drop. 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 a value of 62.7±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. At week 9, there was a concomitant decrease in the leucocrit and lymphocyte percentages accompanied by an increase in the percentage of neutrophils. This was attributed to the stress caused by fighting among these fish as they reached their sexual maturity.

During wound development the thrombocytes increased significantly from a control value of 0.8±0.5% to a value of 10.4±2.2% at the 12 hour attachment (Figure 41E). This value then fell to a somewhat lower value at 2 days attachment. This conforms with the findings of Casillas and Smith (1977) who found that thrombocyte counts increase after stress.

The thrombocytes during wound healing remained at a slightly elevated level throughout the 3 month period (Figure 41F).

During wound development the neutrophilic series increased from a control value of 3.6±1.8% to a value of 15.8±6.3% after 2 days of attachment (Figure 42A). This may have been a sign of the start of the inflammatory response of the fish toward the wound caused by the lamprey.

In wound healing, the neutrophilic series at detachment was 4.7±1.9% and then it made a sharp rise to 31.5±7.2% at 2

Figure 42. Neutrophilic series and constituents during wound development and wound healing in lamprey-attacked fish expressed as a percentage of the white blood cell differential.

(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 neturophil (%) 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.

* The blood smears obtained at ten day lamprey attachment were of poor quality therefore a white blood cell differential was not obtained.

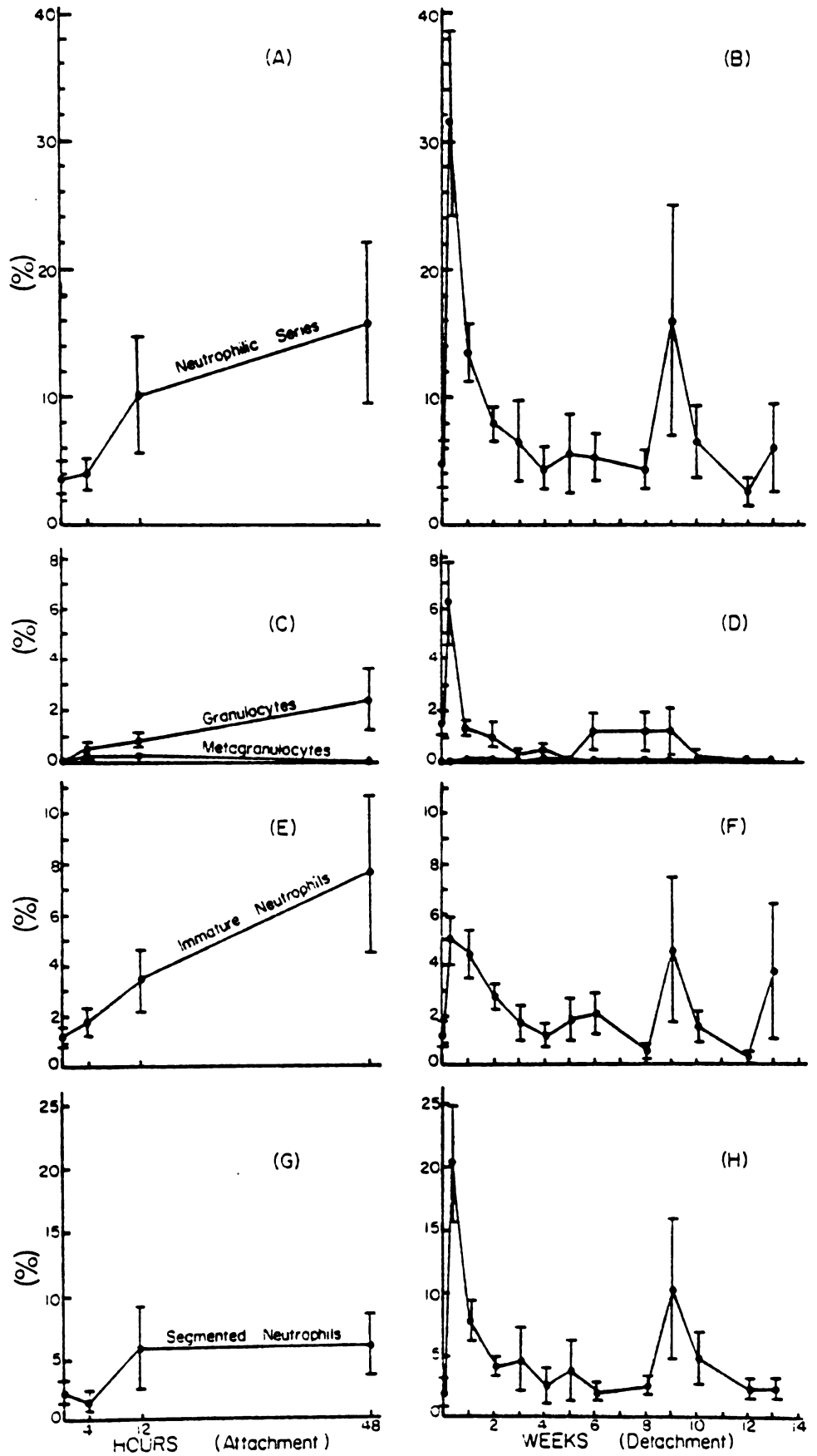
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(%)
Neutrophilic Series
Granulocytes
Metagranulocytes
Immature Neutrophils
Segmented Neutrophils
HOURS (Attachment)
WEEKS (Detachment)

Figure 42

days (Figure 42B). This also happened to be the time when the highest cellular response was noted in the 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 at this time and was an ideal entry area for pathogens. The neutrophilic series remained elevated at week 1 and had a value of 13.4±2.3%.

During wound development the granulocytes, immature neutrophils, and segmented neutrophils increased through the attachment periods, with the immature and segmented neutrophils having the larger percentage present at 2 days attachment (Figure 42C, E and G). The metagranulocytes were present in insignificant numbers.

During wound healing the granulocytes, immature neutrophils and segmented neutrophils increased significantly at 2 days with the segmented neutrophils attaining the highest percentage of 20.3±4.7% (Figure 42D, F and H). This definitely 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.

In the blood data that have been presented, no cells were identified as monocytes, basophils, or eosinophils. 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 which 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 though they reported that neutrophils and metamyelocytes could easily be mistaken unless cytochemical staining methods were used. Basophils and eosinophils have been reported absent 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.

Blood Parameters of Some Mortally Wounded Fish

The blood parameters of some mortally wounded fish are given in Table 3. The hemoglobin, hematocrit and leucocrit values at detachment were as low as 0.2 g/dl, 2.0% and 0.30%, respectively. The red blood cell precursers were as high as 15% in one fish.

White blood cell differentials could only be obtained on the fish which was not near death until 10 days after lamprey detachment. This fish 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

Table 3. Blood parameters of some mortally wounded fish

Lamprey Attachment Time	Hematocrit at detachment (%)	Hemoglobin at detachment (g/dl)	Leucocrit at detachment (%)	RBC Precursers 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.

the greatest percentage of the neutrophilic series. The lymphocytes were 100.0% at detachment, 96.0% at 1 week, and 74.0% at 10 days 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 the percentage of blood volume lost daily since one knows y, the time to its death in days. For instance, if the fish near death after 5 days of lamprey attachment, given in Table 3, was used to find the daily blood volume loss, a value of approximately 50% would be obtained. This is indicative of the great demands on the hemopoietic organs of the fish, since its blood volume would have to be replaced every 2 days. Because the RBC precursers were only 15% of the red blood cells, it is obvious that once the blood reserves from the spleen and kidney are depleted the fish would succumb since the formation of new blood cells is a slow process.

This fish that was near death following 5 days of sea lamprey attachment weighed 402.9 g and was attacked by a lamprey weighing 37.7 g as given in Table 2. So if one uses the same assumptions given earlier, such as the lamprey consuming 11.1% of its body weight per day and the trout having a blood volume equal to 5% of its body weight it is estimated that the fish would lose an estimated 20% of its blood volume per day and would succumb by day 21. This definitely

was not the case as the fish was near death 5 days after attack, thus indicating that some lampreys are more lethal than others. This lamprey would have to consume approximately 27% of its wet weight per day to cause a 50% blood volume loss per day which would have resulted in the fish dying in 5 days.

Farmer et al. (1975) found the maximal food consumption rates of sea lamprey were 20 to 30% of their wet weight per day regardless of their size. They found the hematocrits of dying fish were greatly reduced from the control values of 34.4±1.8% to 1.9±1.7% and the percent moisture of the blood of dying fish increased from 84.5±1.0 to 96.4±1.5%.

Farmer et al. (1975) indicated that trout survival time increased with their weight for lampreys of a given size. They estimated times to death for trout with blood volume losses of 10% or less to be 60 days or longer thus suggesting survival of these individuals. Only trout of 2000 g or greater survive an extended attack by lampreys averaging 50 g, and only those of 4000 g or more for 100 g lampreys. Trout must be greater than 8000 g to survive an extended attack by large sea lampreys of 200 g whose intake is 12% of their body weight per day. They indicated that the lamprey feeding rate and thus trout survival time varies with water temperatures and that the feeding rate of a lamprey feeding on one host over extended periods would increase as the lamprey grew.

Pycha (1970) observed that sea lamprey were selective toward larger trout and found that mortality increased with the size of lake trout in Michigan waters of Lake Superior. For fish of age V or greater, mortality was related to the length of the fish. Lake trout more than 26 or 27 inches long had a high mortality which ranged from 65 to 100% per year due to parasitism by the sea lamprey.

One fish was not near death until 10 days after lamprey detachment. It progressively lost weight from 311.5 g at detachment to 279.9 g 10 days following detachment. The wound area became infected after detachment and a fungal infection was seen at 4 days. Some of the blood data obtained from this fish is presented in Table 3. The hematocrit dropped from 15% at lamprey detachment to 10% at 7 and 10 days following detachment. This drop could be attributed to the extensive hemorrhaging that occurred in the wound area during this time. The number of RBC precursers had risen during this period to 9.6% at 10 days following lamprey detachment. The leucocrit was depressed to a level of 0.44% at detachment which would indicate that its immunological response was somewhat depressed. The white blood cell differential showed 0% neutrophilic series and 100% lymphocytes at detachment. Ten days following lamprey detachment, the neutrophilic series increased to 25% of which most were immature and segmented neutrophils, and the lymphocytes dropped to 74%. The leucocrit at this time also rose to 1.12% thus possibly indicating that instead of a relative

rise in the neutrophils that this rise may have been absolute with the lymphocytes staying depressed because of the increased stress condition at this time. This increase in neutrophils could be attributed to the infected wound during this time.

Hines and Spira (1973) found that mirror carp infected with Ichthyophthirius multifiliis had a sharp lymphocyte drop with a concurrent rise in neutrophil percentages with the overall white blood cell count remaining the same as that of normal carp.

It is therefore evident that fish severely wounded by lamprey are more prone to infections because of the induced anemia and immunological suppression indicated by the depressed hemoglobin, hematocrit, and leucocrit values. A weakened fish in such a stressful situation probably has little chance for survival.

Roth (1972) observed that after intravenous administration of cortisol or cortisone in white suckers, fungus growth was facilitated and thus suggested an impairment of antibody formation and tissue inflammation suppression. Robertson et al. (1963) found that rainbow trout treated with high doses of hydrocortisone had a rapid weight loss and developed infections of the skin. Neish (1977) thought increased levels of plasma corticosteroids in fish increases the chances that Saprolegnia spp. will initiate infections either alone or concurrently with other opportunist parasites.

Mazeaud et al. (1977) observed high levels of corticosteroids in two unstressed male coho salmon with fungus disease. They thought that besides interference with the immunological response to disease organisms that other effects of stress such as the possibility of changes in mucous production may be possible.

Weinreb (1959) found that cortisone injection into rainbow trout resulted in inhibition of wound healing, with less granulation tissue, fewer inflammatory cells and more extensive necrosis.

Lennon (1954) found that blood was usually the most significantly altered of the tissues affected by sea lamprey wounds on fish. Death to most of the fish attacked was attributed to severe hemorrhage which was made worse by their small blood volume.

Lennon (1954) determined the mean blood values for 199 normal white suckers and found that there were 1,159,256 erythrocytes and 3,869 leucocytes per cubic mm and 8.24 g/dl of hemoglobin in whole blood. Post attack blood samples from 119 mortally wounded suckers averaged 189,705 red cells and 8,514 white cells per cubic mm, and a hemoglobin value of less than 3.75 g/dl. Thus the erythrocyte and hemoglobin levels had reductions of 83.6% and at least 54%, respectively, while the leucocytes increased 2.2 times in the injured fish. The host suckers had a mean length of 13.0 inches while the lamprey averaged 12.1 inches long. Their deaths occurred in an average of 59.1 hours.

The leucocrits of the mortally wounded fish of the present study were depressed to low levels thus indicating that the leucocytes were probably in low concentrations at this time. Lennon (1954) found that many dying suckers had white cell counts below normal but on the average there was an increase. He concluded that the gross increase of white cells in the blood, as a defensive mechanism, exceeds the loss of cells due to hemorrhage and thus suckers were able to manufacture leucocytes faster than the cells were removed by the lampreys.

Lennon (1954) observed that twelve dying rainbow trout had erythrocyte counts of 165,167 cells per cubic mm which was 14.9 percent of the mean count of 1,107,500 in 2 healthy fish. The hemoglobin in the blood was reduced by at least 90%. There were 4,052 leucocytes per cubic mm in the wounded trout compared with an average of 7,980 leucocytes per cubic mm in healthy rainbow trout thus indicating a 49.2% drop in white cells. A 48.1% drop in white cells was noted in mortally wounded brook trout. Thus Lennon's white cell decrease for trout is consistent with the present research in that the mortally wounded rainbow trout had depressed leucocrits.

Lennon (1954) thought that white suckers and trout were in mortal danger when their erythrocyte counts fell below 300,000 cells per cubic mm during a sea lamprey attack. He found that fish under attack died in as little as 4 hours whereas others continued to live up to 9 days or longer before their deaths. The host's ability to survive was found

to be dependent on its size and species, the size of the lamprey, the intensity of attack, and the feeding penetration location.

Histopathology of the Kidney and Spleen

The most striking changes occurred in the spleen (Figure 43). Some of the fish exhibited blood congestion in the spleen red pulp regions, especially those of the 10 day wound development group and those of the 2 day wound healing group (Figure 44). The lymphoid (white pulp) region may have released excess numbers of red blood cells into the red pulp region to meet the demand caused by lamprey feeding. The most striking change in the spleen was the depletion of the white pulp region in the fish which had low hematocrits which resulted in a subsequent loss of the once discernible red and white pulp regions (Figure 45). This can be attributed to the excess burden placed on this hemopoietic organ which ultimately could not meet the demand placed on it.

No significant changes in the hemopoietic tissue 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.

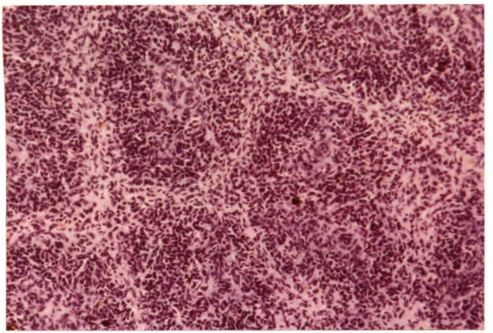

Figure 43. A normal rainbow trout spleen showing the red and white pulp regions
H & E. X100.

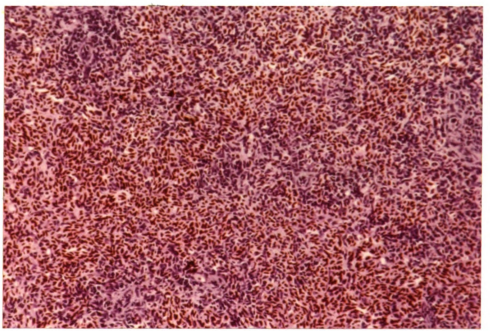

Figure 44. Red blood cell congestion in the red pulp region of the spleen.
H & E. X100.

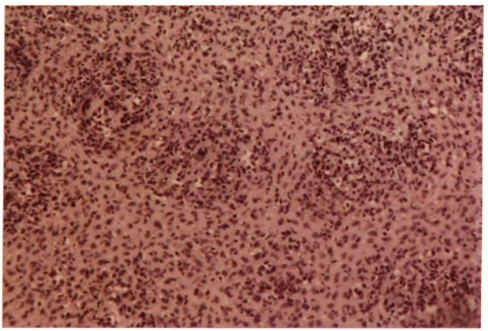

Figure 45. Spleen from a rainbow trout that was near death after five days of sea lamprey attack - note the diminished white pulp regions H & E. X100.

APPENDIX

Table 4. Percent weight and length increases in wound healing fish $\bar{X} \pm$ S.E.

Weeks (Detachment)	Percent Increase in Weight	Percent Increase in Length
2 days	-1.8±0.5	0
1	4.0±1.8	
2	7.5±1.5	0.7±0.4
3	11.6±1.5	
4	14.4±3.0	1.2±0.8
5	16.9±3.2	
6	23.0±3.1	
8	39.4±4.0	
9	42.3±2.7	
10	39.3±3.0	
12	52.9±2.8	
13	59.5±5.6	9.1±0.5

Table 5. Hematocrit, hemoglobin, red blood cell precurser and leucocrit values for wound development and wound healing fish. $\bar{X} \pm$ S.E.

Time	Hematocrit (%)	Hemoglobin (g/dl)	RBC precursers (%)	Leucocrit (%)
		WOUND DEVELOPMENT		
Control	24.1±1.8	6.6±0.7	2.2±0.7	0.97±0.10
4 hrs	27.0±0.8	7.6±0.3	1.5±0.4	0.97±0.09
12 hrs	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
		WOUND HEALING		
Detachment	20.7±1.9	6.0±0.5	5.3±0.9	0.59±0.04
2 days	14.0±3.5	3.7±0.9	6.0±1.1	0.66±0.09
1 wk	19.8±1.3	5.7±0.4	6.9±1.9	1.16±0.09
2 wks	21.5±1.1	5.8±0.4	13.9±3.1	1.19±0.06
3 wks	21.6±1.3	5.4±0.3	16.3±3.6	1.07±0.08
4 wks	22.4±0.9	5.6±0.3	20.9±3.5	1.05±0.10
5 wks	21.6±0.9	5.7±0.4	10.6±3.0	1.06±0.07
6 wks	24.7±0.4	5.7±0.2	10.9±2.3	1.14±0.07
8 wks	25.0±0.7	6.1±0.4	15.4±2.9	1.11±0.09
9 wks	23.3±1.3	5.5±0.3	15.2±1.7	0.91±0.10
10 wks	23.4±1.2	5.4±0.5	10.1±1.4	1.04±0.11
12 wks	26.1±0.9	6.5±0.3	15.9±2.3	1.17±0.07
13 wks	25.7±1.4	5.7±0.2	10.4±4.2	1.25±0.22

Table 6. Components of the white blood cell differential by percent occurrence for wound development and wound healing fish $\bar{X} \pm$ S.E.

Time	Neutrophilic Series	Granulocytes	Metagranulocytes	Immature Neutrophils	Segmented Neutrophils	Lymphocytes	Thrombocytes
			WOUND DEVELOPMENT				
Control	3.6±1.2	0	0	1.2±0.4	2.4±0.9	95.6±1.3	0.8±0.5
4 hrs	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 hrs	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
			WOUND HEALING				
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 wk	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 wks	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 wks	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 wks	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 wks	5.5±3.2	0	0	1.7±0.9	3.8±2.5	89.7±3.5	4.7±1.2
6 wks	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 wks	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 wks	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 wks	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 wks	2.5±1.2	0	0	0.3±0.3	2.3±0.9	92.8±2.1	4.7±1.3
13 wks	6.0±3.5	0	0	3.7±2.7	2.3±0.9	88.7±4.5	5.3±1.7

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