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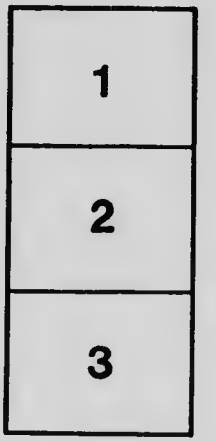

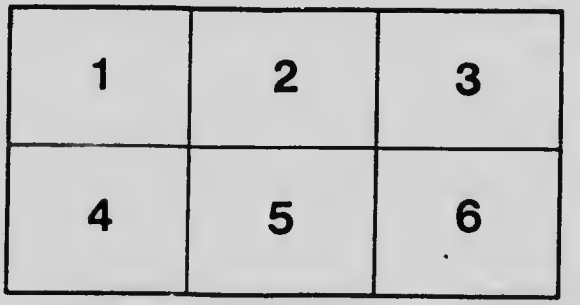

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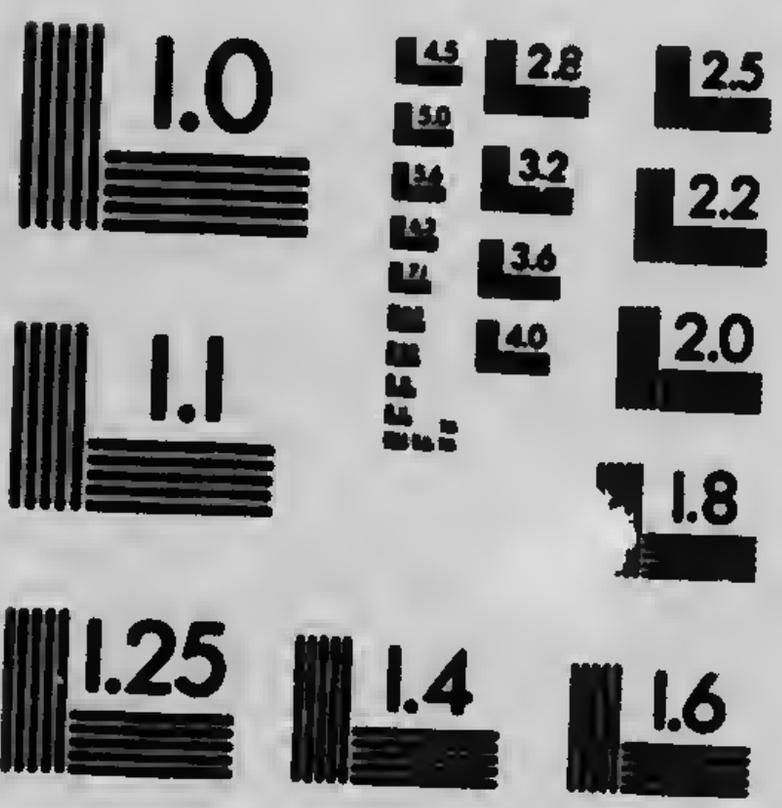

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REPORT

ON

EXAMINATION OF AFFECTED SALMON FROM MIRAMICHI HATCHERY POND, N.B.

By F. C. HARRISON, D.Sc., F.R.S.C., etc., Principal of Macdonald College, Ste. Anne de Bellevue, P.Q.

(With one Half-tone Plate.)

OTTAWA
J. DE LABROQUERIE TACHÉ
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VIII.

EXAMINATION OF AFFECTED SALMON, MIRAMICHI HATCHERY, NEW BRUNSWICK.

On October 11, 1915, I received a telephone message from Dr. A. B. Macallum, Secretary-Treasurer of the Biological Board of Canada, with reference to a diseased condition of the salmon in the hatchery at South Esk, N.B. He also informed me that Dr. Huntsman, of the University of Toronto, was leaving in order to investigate the trouble, and if I thought it wise to do so I could join him and proceed to the hatchery.

I got into telephonic communication with Dr. Huntsman on his passing through Montreal, and after discussing the situation thought it best to remain at the laboratory to examine the diseased fish that Dr. Huntsman would send me in order that I might investigate the disease, for it seemed better to attempt the finding out of the trouble with all bacteriological facilities to hand, which would have been lacking at the hatchery, and which at that time it was impossible to take there.

Retaining Pond at the Miramichi Hatchery, South Esk, N.B.

On October 14, I received a copy of the letter which Dr. Macallum received from the Deputy Minister of the Department of Naval Service, reading as follows:—

> The officer in charge of the Miramichi hatchery, which is located on the South Esk river, a small tributary of the Southwest Miramichi, recently reported that a disease had broken out amongst the salmon in the retaining pond in connection with the hatchery in which the parent fish are placed and retained

until the spawning time comes around. It happened that the Superintendent of Fisheries was in the Maritime Provinces when this information was received, and I had him instructed to visit the pond and look into the matter.

There were on Tuesday of this week somewhat over 2,400 salmon in the pond, between 300 and 400 of which were affected. The disease takes the form of a fungus. The first indication is the removal of the scales from the back of the neck. They are evidently eaten off. Then a white fungus develops, which rapidly spreads down the head to the eyes and makes the fish blind. It subsequently appears on different parts of the body and on the extremities of the fins and tail. The fish diseased were beginning to die, which indicates that they will not last more than a week or ten days after they become affected.

An examination of the pond revealed no reason for any unhealthful conditions. Neither did there seem to be anything through which the water was flowing before it reached the pond to cause it to be unhealthful. Some fish that were in the towing pontoons which had recently been taken from the fishermen's nets to be placed in the pond, were examined, and on a few of them the first stage of the disease above referred to was in evidence.

As it seemed possible that the scales might have been removed from the fish striking the top of the pontoons, one of the fishermen's nets was visited and when lifted there were three salmon and a grilse in it. Two of the salmon were large females weighing about fifteen pounds, and they were perfectly healthy, but the third, a small male weighing 5 or 6 pounds, was apparently affected, as the scales were eaten away from the back of the head and he had an unhealthy appearance.

It would appear from the above that an epidemic has broken out amongst the fish in the river, and in view of the importance of the matter it is desirable that a capable bacteriologist should be immediately sent to the pond to thoroughly investigate the whole matter. I may add that this pond has been in operation for many years and in no instance in the past has any such trouble been experienced. The tide enters the pond, and at each high tide the water is slightly brackish.

I shall be obliged if you will give the matter immediate consideration and wire me whether the Biological Board can at once arrange to send a properly qualified man to investigate the matter. If it cannot, it may be possible for the Department to arrange with that of Agriculture to send an officer from the laboratory at the Experimental Farm here.

N.B.—Since writing the above a report has just been received from the officer in charge of the Port Arthur hatchery, in which he states that a disease, apparently of a similar nature, has broken out amongst salmon trout in the Nipigon river. This is the first time that the department has heard of any such disease there.

A few days later I received a statement from Dr. Huntsman, the main points of which are contained in his report on this outbreak of salmon disease, now being published.

On the arrival of the specimens of fish sent by Dr. Huntsman, they were immediately examined. They arrived in good condition, packed in ice, and were opened in the usual way. After examination of the organs and the flesh near the diseased spots or where the fungus was growing, pieces of the various organs were excised with a sterile knife, and cut open with a second sterile knife, and a portion of the pulp, etc., of the organ removed by means of a sterile platinum loop. In a few cases pieces of the organs were taken out, seized with the forceps and scorched in the flame, and then cut open with a sterile knife and a portion removed to sterile petri dishes. In all cases the material was mixed with beef peptone salt-water agar, and from the various

fish a large number of colonies were isolated. These colonies were lettered and numbered, and besides those here described a large number of other colonies were isolated, which were compared and found similar to those mentioned by letter and number.

FISH No. 1. Appearance normal, with the exception of a few patches of diseased skin around the head. On opening, the organs appeared normal. Plates were made from milt, liver, swimming bladder, kidney, heart's blood. In all cases the material was transferred to sterile petri dishes and beef peptone salt water agar poured over. After the plates had set they were kept at 20°C. Results:—

Milt.—About 60 colonies.

Liver.—About 100 colonies.

Swimming bladder.—Contained a quantity of liquid. Very large number of colonies, too numerous to count.

Heart's blood.—About 300 colonies to the oese. All these colonies were very similar.

Kidneys.—About 90 colonies.

Four species were isolated from this fish, marked A1, A2, A3, A4.
Flesh near diseased skin normal in appearance.

FISH No. 2.—External appearance normal except some bruises with traces of the fungus development near tail and head. On opening, the liver was rather pale in colour, somewhat friable, intestines empty, caeca empty. Right ovary eggs pink in colour; left ovary eggs much darker in colour, almost liver-coloured. Flesh normal and good colour. Same technique. One oese from each of the parts mentioned.

Ovary.—Pink eggs. From one crushed egg 300 or 400 colonies developed. A larger number from the one crushed egg from the dark red left ovary.

Liver.—20 colonies.

Heart's blood.—60 colonies per oese, all practically identical.

Isolations B1, B2, B3, B4.

FISH No. 3.—Exterior appearance normal with the exception of a few small areas discoloured visible in the skin. Flesh normal in appearance. Interior organs apparently normal. Smears from the various organs showed bacteria.

Heart's blood.—About 250 colonies to the oese, all similar.

Eggs.—Innumerable colonies. Two species.

Liver.—20—30 colonies per oese.

Kidneys.—80—100 colonies.

Two isolations—C1, C2.

FISH No. 4.—A large fish; much gelatinous slime around the tail. Some areas of skin affected with the fungus. Flesh beneath appeared healthy. Intestines slightly congested, empty. Liver dark in colour. Eggs salmon pink in colour, apparently normal. Swimming bladder empty. Smears from the heart's blood liver and kidney showed a number of organisms:—

Heart's blood.—30—40 colonies, all similar.

Liver.—10—12, all similar.

Kidneys.—20 colonies, all similar.

Eggs.—About 150 per egg. This is an estimate, as a large growth had occurred in the vicinity of the crushed part of the egg.

One isolation, D1.

FISH No. 5.—Skin between the eyes and the back of the head was bruised and in places dirty white in colour. Microscopical preparations showed the presence of fungus. Flesh normal. All organs normal. Intestines empty. Smears from the milt, liver, heart's blood showed a number of organisms. Plates:—

Heart's blood.—Numerous colonies.

Liver.—40—50 colonies.

Milt.—A few colonies.

Three isolations—E1, E2, E3.

FISH No. 6.—Skin bruised between eyes, fungus present in this area. Flesh normal. Organs normal in appearance. Intestines empty. Eggs, salmon pink in colour. Intestines slightly congested. Smears from heart's blood, liver and egg showed bacteria present. Plates:—

Heart's blood.—About 80 colonies, all similar.

Liver.—30—40 colonies, all similar.

Eggs.—One egg about 200 colonies, all similar.

One isolation, F1.

FISH No. 7.—A large amount of diseased skin from which preparations of the fungus were prepared. Flesh normal. Intestines empty. Organs apparently healthy.

Kidneys.—About 30 colonies, all similar.

Liver.—About 50 colonies, all similar.

Heart's blood.—30—40 colonies, all similar.

One isolation, G1.

FISH No. 8.—Large amount of diseased skin from which fungus growth was easily demonstrated. Liver pale in colour. Ovary deep reddish. Intestines empty. Many whitish eggs in ovary. Spleen normal. Plates:—

Egg.—About 150 colonies to the egg, large masses of bacterial growth near the crushed portion.

Liver.—About 250 colonies.

Heart's blood.—About 150 colonies, all similar.

A number of diseased portions of skin were cut off and examined in a variety of ways. Very good prepartions were obtained by teasing portions of the diseased skin, triturating the material with 40 per cent potassium hydrate. After removal from this reagent they were washed in water and transferred to Lugol solution, or else stained with safranin, eosin, or fluorescein, dehydrated and mounted in balsam. Such teased particles of the skin gave, as a rule, better results than sections.

These preparations show that the fungus was a *Saprolegnia*, and I presume that full particulars of this fungus have been already given by Dr. Huntsman. A very full account of the salmon disease probably caused by *Saprolegnia* is given in the report of the United States Commissioner of Fisheries for 1878, the article having been reproduced from the proceedings of the Royal Society of Edinburgh, written by A. B. Stirling, of the Anatomical Museum of the University of Dublin. A very comprehensive paper by S. Walpole and Prof. T. H. Huxley entitled "Disease among the Salmon of many Rivers in England and Wales" appears in the bulletin of the United States Fish Commission, vol. 1, 1881, and was a reprint of a pamphlet contained in the "21st Annual Report of the Inspector of Fisheries for England and Wales for the year 1881 presented to both Houses of Parliament by command of Her Majesty."

It seemed peculiar that injuries, which appeared at first to be mere abrasions, and which subsequently became infected by the fungus *Saprolegnia*, should have such

a disastrous effect upon the fish as to produce sluggishness and death in the short period of time mentioned by the officer of the hatchery and by Dr. Huntsman, and it therefore seemed important to make a thorough examination of the diseased fish to see if there were other factors producing disease, and to ascertain if the fungus *Saprolegnia*, was a primary or a secondary invader. Unfortunately such investigation was hampered by the fact that no live salmon were available for inoculation, and the only means of ascertaining the pathogenicity of the organisms isolated was to attempt to infect the common gold fish.

During the course of this examination I obtained a publication of the Fishery Board of Scotland entitled "The Life-history of Salmon in Fresh water, Glasgow, 1898," containing a paper by J. Hume Patterson, Assistant Bacteriologist of the Corporation of Glasgow, on "The Cause of Salmon Diseases", and I am indebted to this paper for the methods which were subsequently used for the inoculation of the live gold fish.

Before the gold fish could be inoculated it was necessary to work out in some detail the various organisms which were isolated from the salmon. The principal biological and cultural characteristics of those were as follows:—

A. 1.

A medium sized bacillus with rounded ends, occasionally bent, which occurs singly and sometimes in short chains. Actively motile, stains well with methylene blue, and is gram negative.

Gelatine Plates:—

24 hours, colonies just visible to the naked eye.

48 hours, colonies 2 mm. in diameter, round, with a liquefying centre saucer-shaped. Centre of the colony dense with a mass of deposited bacteria.

With $\frac{1}{6}$ objective edges of the colony seemed slightly fimbriate, and the mass within the centre might be seen moving.

3 days, colonies had grown to between 5 and 9 mm. in diameter, but with similar appearance to that at 48 hours.

4 days, geletine completely liquefied.

Gelatine Stick:—

Growth is best at the top. Line of puncture filiform.

24 hours, Liquefaction begins, extending to the sides of tube and about 2 mm. in depth.

48 hours, growth uniform, line of puncture a cloudy area 10 mm. in diameter with small outgrowths into gelatine forming a cloudy cylinder. At the surface liquefaction is stratiform to a depth of 4 mm.

3 days, the growth has increased, stratified liquefaction extended to a depth of 7 mm. and the cloudy area looks like a saccate cylinder.

8 days, liquefaction to a depth of 8 mm.

10 days, there is a distinct dark stratum underneath the liquefied area.

13 days, very slight increase.

Beef Peptone Agar, 48 hours:—

Colonies 1 - 2 mm. diameter, round, raised, entire edge, glistening white appearance. With the $\frac{1}{6}$ objective the edges were entire, colonies dense, and grandular with a narrow clear margin.

3 days, colonies 2 - 5 mm. diameter, round, more massive and dense, convex, whitish to light brown in centre.

Sloped Beef Peptone Agar, Blood Heat (37.5):—

Little change after three days' growth.

The organism grew fairly well at blood heat.

24 hours, spread over about half the sloped surface.

48 hours, growth denser, spreading, flat, glistening, smooth, semi-opaque, whiteish. No further change.

Glucose Agar Slope:—

24 hours, at room temperature, smooth, vigorous, whitish, moist and spreading. Cloudiness near the growth.

5 days, colony more cloudy, considerable gas production and the column of agar is burst apart in the middle.

Glucose Agar Stick:—*24 hours*. Growth vigorous over surface and pronounced cloudiness from the surface to a depth of 10 mm.

48 hours. Increase in growth and a few gas bubbles appear on the line of puncture.

No further change occurs.

Beef Broth:—

24 hours, strong, cloudy.

3 days, much heavier. Sediment flocculent.

7 days, yellowish-green appearance in the upper layer otherwise no change.

Dunhams's Solution:—

The organism grew well in Dunham's solution, and at the end of 5 days at room temperature was tested with Ehrlich test, allowed to stand 20 minutes and the results then recorded. This organism was negative to this test. No Indol.

Milk:—

24 hours, no change.

3 days, coagulated with extrusion of slight amount of whey.

5 days, curd has become firmer, and a cheesy smell developed.

7 days, slightly more whey extruded;

No other change, although observed for some twenty days.

Litmus Milk:—

24 hours, no change.

48 hours, no change in constituency, but colour is changed to avellaneous.[1]

5 days. Colour uniform, slight digestion with separated whey, soft curd, yellowish ring around glass, smell disagreeable.

3 weeks.—Curd still undigested, whey yellowish, yellow ring, curd avellaneus, few gas bubbles on shaking.

Potato:—

24 hours. Moderate, dry, slightly raised, cream-coloured growth.

48 hours Increase of growth, dry, raised, slightly rugose, cream-yellow colour.

6 days. Abundantly raised, massive, rugose growth, cream colour at margins and pinkish on top. Odour unpleasant and slightly pungent, resembling that on milk.

3 weeks. No change.

A. 2.

Small bacillus with rounded ends, short, often in pairs, actively motile, stains well with methylene blue, and is gram negative.

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[1] *Chromotaxia seu Nomenclator Colorum*. P. A. Saccardo.

Gelatine Plates:—

24 hours. Colonies just visible to the naked eye.

48 hours. Colonies have attained a size of 2-3 mm. in diameter; round, saucer-shaped. In the centre a dense mass of deposited bacteria with liquefying area around. With ⅔ objective interior of the colony is glumose. Edges clearer, but less distinct than A. 1.

3 days. In moderately seeded plates there is complete liquefaction.

Gelatine Stick:—

24 hours. Growth uniform. Line of punctures a cloudy area 5 mm. in diameter along line. Liquefaction begins in 24 hours, extending to sides of tube and 3 mm. in depth.

48 hours Increase in growth with similar appearance, and stratified liquefaction to a depth of 5 mm. Liquefaction gradually increases.

4 days. 10 mm. deep and the remainder of the tube saccate liquefies.

6 days. Liquefaction to a depth of 4 cm.

10 days. Liquefaction of the gelatine in the tube complete.

Beef Peptone Agar:—

48 hours at room temperature. Colonies 1-2 mm. in diameter, raised, glistening, whitish colony by reflected and greenish opalescent by transmitted light. With ⅔ objective edges entire, centre granular with a clear hyaline margin all around.

3 days. Not much increase in size, but more in density. Colony becoming whiter and more convex, somewhat resembling a yeast colony.

Beef Peptone Agar, at 37°C.:—

Very slight growth at *24 hours,* after which there was no further growth.

Glucose Agar Slope:—

24 hours. Abundant, flat, slightly spreading, smooth, moist, whitish growth.

No further change noticed until about second week, when the agar becomes brownish beneath the slope.

Glucose Agar Stick:—

24 hours. Growth filiform on surface, thin and spreading. Not characteristic.

48 hours. Gas bubble on surface and below. Afterwards no further change.

Beef Broth:—

24 hours, strong clouding, which increases, with abundant sediment.

No further change.

Dunham's Solution:—

5 days, at room temperature; tested with Ehrlich's reagents; allowed to stand for 20 minutes and then recorded. No Indol.

Milk:—

24 hours. No change.

3 days. Coagulated with extrusion of slight amount of whey.

5 days. Curd becomes firmer, and cheesy smell develops.

Amount of whey increases up to seventh day, after which there is no further change.

Litmus Milk:—

24 hours. No change.

48 hours. No change in colour or consistency; on shaking numerous small gas bubbles appear and form a foam on surface.

6 days. Coagulated, moderately firm curd, liliacinus in colour. About a quarter of the tube is whey, and much darker in colour (atro-violaceus).

8 weeks. There is a reddish ring at the surface, considerable digestion, whey occupying three-quarters of the tube, isabellinus in colour. Curd flocculent, avellaneus; odour slightly cheesy.

Potato:—

24 hours. Growth moderate, filiform, slightly raised, cream-yellow colour. This increases, and in

6 days growth is moderate, raised, rugose, moist, shiny; dirty cream-yellow, darker in centre where growth is most massive.

8 weeks. No further change.

A. 3.

Medium-size bacillus with rounded ends, resembles A. 1 in appearance. Active motile, stains well with methylene blue, and is gram negative.

Gelatine Plates:—

24 hours. Just visible to the naked eye. Growth rapid.

48 hours. Colonies are 2-5 mm. in diameter, round. Liquefaction saucer-shaped, inner ring dense, caused by deposited bacilli. With ⅔ objective the edges of the colonies are fimbriate centre grumose and flocculent. Masses of the bacteria can be seen in movement.

3 days. Colonies increase to 12 mm. in diameter, saucer-shaped liquefaction, whitish in centre, more transparent at the margin. To the naked eye the edges are entire, but with a microscope slightly fimbriate. There is a cheesy smell on opening the plates.

4 days. Plates are liquefied.

Gelatine Stick:—

24 hours. Resembles A. 1, but slightly less growth.

48 hours. Line of bacteria is filiform, smooth on surface. Liquefaction stratiform, 4 mm. deep. Liquefaction continues.

10 days. Liquefaction is 1 cm. deep with medium beneath darker in colour, but clear.

Agar Plates:—

48 hours. Colonies are 1-3 mm. in diameter, round, raised, yellowish-white. With ⅔ objective edges are entire, dark in centre, granular, gradually becoming lighter to margin, which is clear.

3 days. Colonies are round, white, edges entire, brownish in centre. Convex.

4 days. No change.

Agar Slope, 37°C.:—

24 hours. Very slight growth, filiform.

7 days. No further change.

Glucose Agar Slope, 20°.—

A spreading, flat, white, shiny growth; agar beneath very cloudy. Cream yellow. No gas.

Glucose Agar Stick:—

Growth filiform, spreading; cream colour at centre, lighter at margins. Cloudy to half-way down the agar.

Beef Broth:—

24 hours. Clouding moderate. Sediment.
3 days. Growth heavier, slight pellicle.
5 days. Ring and pellicle.
7 days. Yellowish-green colour in upper layers.
Subsequently no change.

Dunham's Solution:—

Grown for five days at room temperature, tested with Ehrlich test, allowed to stand 20 minutes and then recorded. No Indol.

Milk:—

Fifth day. No change until the fifth day, when there is coagulation with soft curd, cheesy odor. Curd gradually becomes harder and the whey greenish in colour. Digestion takes place to about half the volume.

Litmus Milk:—

The colour is gradually bleached and in *48 hours* is avellaneus.
5 days. Coagulation takes place in 5 or 6 days, a soft, fine curd which gradually digests. Blue ring at the top; separated whey is isabellinus in colour.
3 weeks. Greenish-blue colour; whey thick, curd avellaneus, odour unpleasant.

Potato:—

24 hours. Growth moderate, raised, filiform, cream-yellow in colour.
48 hours. Growth becomes dirty and ochraceus, slightly rugose. Growth gradually changes to ferrugineus in colour.
3 weeks. No change

A. 4.

A small bacillus, short, rather stout, with rounded ends. In appearance resembles A. 2. Actively motile, stains well with methylene blue, and is gram negative.

Gelatine Plates:—

24 hours. Just visible to the naked eye.
48 hours. Colonies punctiform (less than 1 mm.) white and glistening, with $\frac{2}{3}$ objective they are seen to be round, with entire edges, and granular.
3 days. Colonies slightly punctiform, white, glistening, convex, capitate. With $\frac{2}{3}$ objective edges entire and granular.
No further change.

Gelatine Stick:—

24 hours. Growth unifrom, line of bacteria filiform.
48 hours. Growth filiform to villous. Four gas bubbles on line of bacteria.
3 days. There is more growth. Line of bacteria villous to papillate.
10 days. Slight depression at the point of puncture may be noticed, but no liquefaction.
13 days. Liquified area around the line of puncture.

Agar Plates:—

48 hours. Colonies are filiform, glistening, raised. With ⅛ objective the colonies are round, dense in centre, and granular, clearer at margin, edges entire.

3 days. Colonies slightly larger, opalescent, white.

No further change.

Gelatine Agar Slope at 37° C:—

Little, if any, growth observed. Continuous observation for 7 days.

Glucose Agar Slope:—

Growth moderate, moist, shiny, slightly raised, whitish.

3 weeks. Agar is brown beneath the slope.

Glucose Agar Stick:—

Growth filiform, thin surface, growth spreading. Gas bubbles along line of puncture.

No further change except the agar becomes brown beneath the surface to a depth of 1-2 cm.

Beef Broth:—

24 hours. Slight clouding and sediment.

3 days. Clouding and sediment increase slightly.

No further change.

Dunham's Solution:

Grown for five days at room temperature, tested with Ehrlich test, allowed to stand 20 minutes and then recorded. Indol positive.

Milk:—

5 days. No change visible.

6 7 days. On shaking tube a gassy foam rises to the surface.

10 days. Milk had coagulated, hard curd, whitish whey.

Litmus Milk:—

No change in appearance in *24 hours.*

48 hours. Abundant gas which rises to the surface in small bubbles. This was noticed each day up to the sixth day, and the foam was very heavy. The milk gradually coagulates and forms a blue ring down one side of the tube, remainder is a firm curd adhering to the tube. Bleached cream colour.

Potato:—

24 hours. Moderate growth, filiform, slightly moist, cream coloured.

48 hours. Becomes slightly rugose.

6 days. Growth slight, slightly raised, and a dirty yellow (melleus).

3 weeks. No further change.

B. 1.

This organism on examination was found to resemble in all respects A. 1.

B. 2.

A small size bacillus about 1½ times as long as wide, rounded end, frequently in pairs. Actively motile, stains well with methylene blue, negative with gram.

Gelatine Plates:—

24 hours. Visible to the naked eye.
48 hours. Punctiform, colony raised, glistening, whitish. ⅔ objective shows round, dense, granular colony, entire edges.
No further change.

Gelatine Stick:—

24 hours. Growth uniform, round, filiform; no liquefaction.
48 hours. Growth uniform, no liquefaction to surface.
3 days. Slight depression at the boint of bacteria. No liquefaction.

Agar plates:—

48 hours. Uniform, 1 m.m. in diameter, round, glistening, colony. With ⅔ objective round, dense, shading to lighter; granular, edges entire.
3 days. Colonies are glistening and bluish white.
No further change.

Agar slope 37° C.:—

7 days. Very slight growth, one or two small colonies appearing on the surface but otherwise no change.

Glucose Agar Slope:—

Moderate growth, spreading, flat, moist and whitish.
48 hours. A few gas bubbles appear and slight increase in growth.
3 weeks. Agar is brown underneath the slope.

Glucose Agar Stick:—

Filiform, slight growth on surface, gas bubbles along line of puncture.
No further change except for browning of the agar underneath the surface.

Beef broth:—

24 hours. Moderate growth, moderate sediment.
3 days. Growth slightly heavier.
5 days. Clearing.
No further change.

Dunham's Solution:—

Grown for five days at room temperature, tested with Ehrlich test, allowed to stand 20 minutes and then recorded. Indol positive.

Litmus Milk:—

24 hours. No change.
48 hours. A fine foam on the surface when tube is shaken. Colour liliaceous, no coagulation.
6 days. Much gas in foam form. No coagulation. Colour liliaceous. Colour gradually bleaches. Blue ring forms on surface. Bluish whey but little digestion.

Potato:—

24 hours. Filliform, dry, raised, colour niveus.
6 days. Growth becomes slightly raised and more massive.
3 weeks. No change.

B. 3

Resembles in all respects *A. 4.*

B. 4.

Resembles in all respects *A. 4.*

C. 1.

Small to medium bacillus about twice as long as broad, slightly rounded ends. Actively motile, stains somewhat unevenly with methylene blue, gram negative.

Gelatine Plates:—

24 hours. Colonies visible to the naked eye.
48 hours. Colonies punctiform, round, white, raised and glistening. ⅔ objective round, evenly dense and granular with entire edges.
No further change.

Gelatine Stick:—

Growth uniform, line of puncture filiform, 4 gas bubbles along line of puncture.
10 days. Depression at the point of puncture.
18 days. Line of bacteria has liquefied.

Agar Plates:—

48 hours. Colonies are punctiform, 1-1½ mm. in diameter, round, raised, white, glistening.
With ⅔ objective colonies are round, dense in centre, clear margins, granular, entire edges.
No further change.

Agar, 37°C.:—

24 hours. Moderate growth, flat, slightly spreading, smooth and translucent.
No further change.

Glucose Agar Slope:—

Flat, moist, spreading, whitish growth, few gas bubbles.
No further change except browning of the agar beneath surface.

Glucose Agar Stick:—

24 hours. Filiform, growth spreading on surface.
48 hours. Few gas bubbles along line of puncture.
No further change.

Beef Broth:—

24 hours. Moderate clouding, flocculent, abundant sediment.
5 days. Clearing.
No further change.

Dunham's Solution:—

Grown for five days at room temperature, tested with Ehrlich test, allowed to stand 20 minutes and then recorded. Indol positive.

Litmus Milk:—

24 hours. No change.
48 hours. Slight amount of gas, colour somewhat lighter, no coagulation.
6 days. Much gas in foam form. No. coagulation. Colour liliaceous.
Subsequently milk coagulates, blue ring, surface clear, whey on one side, curd adhering to two-thirds of the tube; bleached to a cream colour and of firm consistency.

Potato:—

Slightly raised, moderate growth, cream-yellow.
3 weeks. No further change.

D. 1.

Medium-size bacillus, with slightly rounded ends, actively motile, stains well with methylene blue, gram negative.

Gelatine Plates:—

Colonies visible to the naked eye in *24 hours.*
48 hours. Uniform, round, white, glistening colony; ⅜ objective round, granular, dense to the edge, edges entire.
3 days. Colonies become more dense. Convex.
No further change.

Gelatine Stick:—

48 hours. Line of puncture is villous. Slight softening of the gelatine on the surface.
Subsequently growth along line of puncture becomes villous to papillate, softening gradually extending along line of puncture.

Agar Plates:—

48 hours. Punctiform to 1 mm. in diameter, round, white, raised, glistening colony; ⅜ objective colonies round, dense in centre to clear margin, granular, edges entire.
3 days. Slight increase in sizes; otherwise no change.

Agar slope, 37° C.:—

No growth at this temperature.

Glucose Agar Slope:—

Moist, flat, spreading, whitish growth. Agar becomes brown beneath the growth, but no further change.

Glucose Agar Stick:—

Line of puncture filiform, spreading on surface, three or four small bubbles appear in *48 hours* and slight increase in growth; otherwise no change except browning under growth.

Beef Broth:—

24 hours. Growth moderate, sediment moderate and flocculent.
5 days. Clearing.
No further change.

Dunham's Solution:—

Grown for five days at room temperature, tested with Ehrlich test, allowed to stand 20 minutes and then recorded. Indol positive.

Litmus Milk:—

24 hours. No apparent change, but on tapping the tube small gas bubbles rise to the surface.
48 hours. Gas more pronounced. Colour liliaceous.
6 days. Foamy gas. No coagulum. Colour liliaceous.
3 weeks. Blue ring on surface cleared away along one side, remainder firm curd adhering to the tube. Bleached cream colour.

Potato:—

Moderate growth, raised, rugose, waxy, cream yellow in colour.

E. 1., E. 2. and E. 3.

Resemble *A. 1.*

F. 1.

Medium size bacillus with rounded ends. Actively motile.

On staining with methylene blue there are two or three dark granules in most of the organisms. Gram negative.

Gelatine plates:—

24 hours. Just visible to the naked eye. Round, white, glistening, ⅛ objective brown, edges entire, granular.

Subsequent liquefaction.

Gelatine Stick:—

Growth uniform, line of puncture filiform, growth becomes slightly heavier and on the 6th day there is a slight liquefied depression.

10 days. Liquefaction is infundibuliform.

13 days. Complete liquefaction.

Agar Plates:—

Agar slope, 37°:—

Very slight, if any, growth (7 days).

Glucose agar slope:—

Filiform, non-spreading growth.

Glucose Stick:—

Filiform growth, nothing on the surface.

6 days. Slightly heavier, subsequently no change.

Beef Broth:—

Slight clouding, flocculent sediment.

3 days, clearing.

No further change.

Dunham's Solution:—

Grown for five days at room temperature, tested with Ehrlich test, allowed to stand 10 minutes, and then recorded. Very weak Indol.

Litmus Milk:—

6 days. No change visible until 6th day, when colour becomes darker. This increases.

6 weeks. Colour is atrocyaneus. There is progressive digestion without coagulation.

Potato:—

Whitish growth restricted and filiform.

3 weeks. No further change.

-. I.

Medium size moderately thick bacillus with rounded ends, very considerable variation as to size, actively motile, stains well with methylene blue, gram negative.

Gelatine plates:—

24 hours. Just visible to the naked eye.
48 hours. Punctiform.
8 days. 1 - 5 mm. in diameter, round, saucer-shaped, liquefaction. Whitish in colour, most dense near centre. Radiating lines like the spokes of a wheel from the centre consisting of deposited bacteria. With ⅛ objective edges are entire and interior granular to grumose.
4 days. Plates have liquefied.

Gelatine Stick:—

48 hours. Liquefaction heavier, 6 mm. in depth. This increases and is stratiform to saccate. In ten days tube is completely liquefied.

Agar Plates:—

Agar slope, 37°:—

Very slight growth, in 24 hours.
48 hours. More abundant growth, spreading, flat, glistening, semi-opaque.
3 days. Slightly heavier.
No further change.

Glucose agar slope:—

Moist, white, spreading, smooth, Gas in condensation water.
3 weeks. Cream-yellow colour at the base of the slope, and centre of surface growth.

Glucose Stick:—

Filiform, slightly spreading on surface, 3 or 4 gas bubble along line of puncture.
No further change.

Beef Broth:

24 hours. Strong, cloudy, moderate sediment.
3 days. Pellicle over entire surface.
7 days. Yellow-cream colour in the outer layers.
No other change.

Dunham's Solution:—

Grown for five days at room temperature, tested with Ehrlish test, allowed to stand 20 minutes, and then recorded. Indol very strong production.

Litmus Milk:—

48 hours. Colour is lighter.
2 days. Alkaline digestion commences.
6 days. Almost complete digestion, remaining curd, in fine particles, dirty violaceous in colour. Whey ¾ of tube. Semi-transparent and avellaneous in colour, no odour. Blue ring at surface.

Potato:—

24 hours. No apparent change.
48 hours. Slight growth, filiform, yellowish.
6 days. Moderate growth, slightly raised, moist on the moist part of potato and dry at the top, ferruginious in colour.
3 weeks. Colour is redder, otherwise no further change.

H. I.

A short to medium stout bacillus, actively motile, stains well with methylene blue, is gram negative.

Gelatine Plates:—

24 hours. Just visible to the naked eye.
4 days. Punctiform, later liquified.

Gelatine Stick:

Growth uniform, filiform.
48 hours. Slightly liquefying, 2 mm. in depth, stratiform. Liquefaction increases and is slightly saccate with flocculence.
10 days. Liquefaction becomes infundibuliform.
15 days. Whole tube is liquefied.

Agar Plate:—

48 hours. Round, uniform, glistening, colony. With 1 objective round, edges slightly erose. Slightly granular colony.
3 days. Colony becomes more massive and bluish white; otherwise no further change.

Agar Slope, 37°:—

Very slight growth, one or two colonies. Increases along line of puncture.
7 days. No further change.

Glucose Agar Slope:—

Thin, translucent, moist, film in 24 hours. No further change.

Glucose Stick:—

Filiform. No surface growth.

Beef Broth:—

24 hours. Slight clouding, flocculent and abundant sediment.
3 days. Clearing.
7 days. No further change.

Dunham's Solution:—

Grown for five days at room temperature, tested with Ehrlish test, allowed to stand 20 minutes, and then recorded. Indol very strong.

Litmus Milk:—

24 hours. No change.
48 hours. Tubes become darker in colour, atro-violaceous. No coagulation. Subsequently there is gradual digestion. Whey first with a violet shade, throughout, which gradually concentrates as a deep blue ring on top, and curd becomes semi-transparent, isabellinus in colour, and thick but not viscous. A little undigested curd at bottom of tube. (3 weeks.)

Potato:—

24 hours. Very slight growth.
4 days. Growth moist, slightly raised, smooth. Colour brown, light testaceous.
3 weeks. Colour changes somewhat between rosaceous and testaceous. No further change.

SUMMARY OF CHARACTERS.

	Diam. of 1 m.	Chains.	Spores.	Capsules.	Motile.	Gram.	Broth. Cloudy.	Broth. Ring.	Broth. Pellicle.	Broth. Sediment.	Agar. Shiny.	Agar. Dull.	Agar. Chrom.	Gel. Plate. Round.	Gel. Plate. Liquef.	Potato. Med.	Potato. Colour.	Potato. Grows at 37° C	Milk. Acid curd.	Milk. Rennet curd.	Milk. Casein ppt.	Milk. Gas.	Indol.	Gas in Gluc. Agar.
A 1.....	–	+	–	–	+	–	+	–	–	+	+	–	–	+	+	+	+	+	–	–	–	+	–	+
A 2.....	–	+	–	–	+	–	+	–	–	+	+	–	–	+	+	+	+	+	–	–	+	+	–	+
A 3.....	–	+	–	–	+	–	+	+	+	+	+	–	–	+	+	+	+	+	–	–	+	+	–	+
A 4.....	–	+	–	–	+	–	+	–	–	+	+	–	–	+	–	+	+	–	–	+	+	–	–	–
B 2.....	–	+	–	–	+	–	+	–	–	+	+	–	–	+	–	+	+	–	–	+	–	+	–	+
C 1.....	–	+	–	–	+	–	+	–	–	+	+	–	–	+	–	+	+	+	–	+	+	+	+	+
D 1.....	–	+	–	–	+	–	+	–	–	+	+	–	–	+	–	+	+	+	–	+	+	±	+	+
F 1.....	–	+	–	–	+	–	+	–	–	+	+	–	–	+	–	+	+	–	–	+	–	+	+	+
G 1.....	–	+	–	–	+	–	+	–	–	+	–	+	–	+	+	–	–	–	–	+	+	–	+	–
H 1.....	–	+	–	–	+	–	+	–	+	+	+	–	–	+	+	+	+	+	–	+	+	–	+	+
B Salmonis pestis.	?	+	–	–	+	–	+	–	+	+	+	–	–	+	+	+	+	–	+	–	+	–	..	–

CHARACTERISTICS OF THE MICRO-ORGANISM (*Bacillus salmonis pestis* Patterson).

Morphological Characters.

A short, thick bacillus with rounded ends, varying in length, occurring singly and in pairs lying end to end. Actively motile, non-spore-bearing, does not stain with Gram's method, grows rapidly and profusely at the room temperature, but shows little or no growth at 37° C., and is killed at this temperature in about six days.

The organism exposed to a mixture of ice and salt for a week not only survived that low temperature, but grew profusely while in the mixture. Involution forms were only observed in glucose media. It appears to be a strict aerobe. Pathogenic to fish, non-pathogenic to frogs, mice, and guinea-pigs.

Cultures.—Room Temperature.

Gelatine Plates.—In about three days small, greyish, pin-point colonies appear, with a ring of liquefaction around them of a transparent greyish colour, which rapidly increases, the plate becoming completely liquefied in about 36 hours after their appearance. The dense pin-point centre and transparent area of liquefaction around is markedly characteristic of the bacillus, together with the very rapid liquefaction of the gelatine.

Gelatine Stab.—Profuse growth along needle track at the end of 18 hours, which gradually increases and rapidly liquefies the gelatine.

Carbol Gelatine, 1 per cent Stab.—Slight growth in 18 hours along needle track, which gradually liquefies the gelatine.

Carbol Gelatine, ·05 per cent Stab.—The growth is more profuse.

Carbol Gelatine, ·03 per cent, Stab.—Very profuse growth.

Room Temperature.	37° C.
Agar Streak :— Dense, profuse, cream-coloured moist shining growth along needle track in 18 hours, with irregular margin, which gradually spreads over the surface of the agar.	Growth barely visible.
Agar Smear :— Small pin-point cream-coloured colonies at the end of 18 hours with irregular spreading transparent margins.	" "
Agar Glucose Stab :— Profuse cream-coloured growth along needle track for about half an inch at the end of 24 hours, spreading on the surface. The agar gradually *becomes cloudy from the surface and parallel to it, and extends for about half an inch down the media. No gas production.*	" "
Agar Glucose Plate :— Cream-coloured colonies with moist shining surface and *white cloudiness around each Colony.*	" "
Blood Serum :—	
Bouillon :— At the end of 18 hours the bouillon becomes cloudy throughout, with a marked skim on the surface and clinging to sides of tube, with a slight deposit at the bottom.	No perceptible growth.
Bouillon (Glucose) :— Similar to ordinary bouillon, but growth much more profuse..	Very slight growth.
Bouillon Taurocholate Glucose :— Slight growth, turning the media slightly red. No gas formation.....	No growth.
Litmus Milk :— In about 48 hours there is a distinct acid reaction, which gradually increases, and in about seven days the milk becomes coagulated and gradually digested.	No perceptible change.
Peptone Water :— Marked cloudiness throughout at the end of 18 hours. Gives no indol reaction.	Very slight cloudiness at the end of 48 hours. Gives no indol reaction.
Potato :— Very profuse yellowish brown growth at the end of 18 hours, raised on the surface of media like blisters, with moist shining surface.	Very slight growth in 48 hours.
Agar (Anaerobically) :— No growth...	No growth.

The organism also withstands the effect of ordinary water, sterile water and *sea-water* for a considerable time, as flasks of those inoculated with it and kept at the room temperature for over a month gave profuse growths when reinoculated on agar. It does not, however, survive more than a week in distilled water. It also keeps well on sub-cultures, as tubes of agar inoculated from sub-cultures about a year old gave profuse growths in about 18 hours.

The chief characteristics of the bacillus are those:—

Actively motile, non-spore-bearing bacillus.
On sub-culture it grows profusely in 18 hours at the room temperature.
On sub-culture it grows profusely when exposed to 0 deg. C. for a week.
Shows *little or no growth at 37° C.*
Is *killed at 37° C.* (98·6° F.) in about six days.
Liquefies gelatine with extreme rapidity.
Coagulates and digests milk.
Forms a cloudiness in glucose agar in the neighbourhood of the growth.
Grows well in sea water.
Strict aerobe.
Involution forms only observed on glucose media.
Does *not* stain with Gram's method.
Pathogenic to fish.
Non-pathogenic to frogs, mice, and guinea-pigs.

CONCLUSIONS.

(1) The fungus *Saprolegnia ferax* is not the cause of the salmon disease.

(2) The disease is due to the invasion of the tissues of the fish by a special bacillus (*Bacillus salmonis pestis*).

(3) The bacillus gains access through abrasion or ulceration of the skin, and the disease is apparently not contracted when the skin of the fish is in a healthy state.

(4) *Bacillus salmonis pestis* can be transmitted from dead diseased fish to other dead fish in the same water.

(5) *Bacillus salmonis pestis* can be transmitted from dead fish to living fish in the same water, and since dead fish are a suitable nidus for the growth of the bacillus, it is obviously desirable to have all dead fish removed from the river immediately they are observed, and burned, as by simply burying, the germ is left in a condition to be again carried into the stream.

(6) The fact that the bacillus grows profusely when placed in a freezing mixture of ice and salt, while a temperature of 37°C. soon destroys it, shows that the cold season is more favourable to its growth.

(7) Fish akin to salmon are more susceptible to the disease than others, as rainbow trout, river trout, and sea trout when attacked succumbed in from two to four days, while dace and gold-fish died in about 18 and 35 days, respectively.

(8) *Bacillus salmonis pestis* grows well in sea water, whereas *Saprolegnia* does not grow at all; therefore a diseased salmon entering the sea, and returning to the river apparently free from fungus, cannot be said to be free from the disease.

GOLD-FISH EXPERIMENT.

Late in November a number of gold-fish were purchased and placed in a large tank in one of our laboratories. The change of water resulted in a few dying, so to avoid any errors due to management we kept them for a month before inoculation. They were then removed from the aquarium and two fish were placed in each of eight large museum jars, and kept thus for another week. The water was changed every third day, and the fish fed every alternate day.

The inoculation was carried out in the following manner: The fish was taken out with the hand and the top of the head and part of one side near the gills gently rubbed with sandpaper until there was a slight effusion of blood, and this abraded area was then rubbed with a platinum oese of 3 mm. charged with material taken from a 24-hour-old agar slope culture. A separate piece of sandpaper was used for each fish. Several loopsful of the culture were added to the water of each jar.

In this way organisms A1, A2, A3, A4, B1, B2, C1, D1, E1, F1, G1 were inoculated in duplicate, and four fish were rubbed with sandpaper but not inoculated. The fish were observed daily, and the inoculated water was changed on the third day.

The control fish rubbed with sandpaper and not inoculated are still alive, and of the inoculated fish one in each of the jars inoculated with A, A2, B2, C1, and D1, died 22, 30, 34, 27, 4 days after inoculation.

Bacteriological examination was made of these fish, but in no case was I able to obtain from the dead fish the organism which was inoculated. Evidently these organisms were non-pathogenic to gold-fish. One fish in each of the jars from which the dead fish were taken remains alive, and, at the time of writing (May 10) appear quite normal. Of course there is the possibility that some of the organisms isolated might be pathogenic for salmon and not for gold-fish.

Patterson states with reference to his *B. salmonis pestis* that:—

> "Dace inoculated with this bacillus died as the result of inoculation in from two to seven days. Dace, river trout, sea trout and gold fish inoculated

with *Saprolegnia* remained healthy. Dace, sea trout and one gold fish inoculated with *Saprolegnia* and *B. salmonis pestis* died in various periods of time (2 to 18 days) except the gold fish which died after inoculation and showed signs of the fungus on the gill covers. No attempt was made to make cultures from the dead gold fish."

Patterson concludes that:—

"*Saprolegnia* grows on live fish in the presence of the organism, which breaks down the superficial tissue and forms a suitable nidus for the fungus to grow on."

I had no *Saprolegnia* to try similar experiments.

The difficulty of obtaining and keeping fish for experiments in a laboratory unequipped for such work, and the difficulty because of lack of laboratory equipment to carry out experimental work at the hatchery, will have to be overcome before any decisive experiments can be undertaken.

It is, however, significant that all organs apparently healthy in the salmon examined contained bacteria in large numbers, and of comparatively few species, and I am unable to state or find in any literature or obtain information as to the bacterial content of the normal organs of fish, or how soon after death, and to what extent, these organs are invaded by bacteria. Very large numbers of bacteria were found in the eggs from a number of the fungus-infected salmon, and under normal conditions one would scarcely expect to find so many bacteria present.

All that can be stated at present is that Patterson's organism, *B. salmonis pestis*, was not found, and that the large number of bacteria present accompanying the *Saprolegnia* may have some pathogenic role, but the rules of proof (Koch's postulates) would have to be worked out where fish, the means of keeping them, and laboratory facilities are provided.